Suppressive effect of ascophyllan HS on postprandial blood sugar level through the inhibition of α-glucosidase and stimulation of glucagon-like peptide-1 (GLP-1) secretion

Takasi Okimura[1], Zedong Jiang[2], Yan Liang[3], Kenichi Yamaguchi[3], Tatsuya Oda[3 †]

[1]*Research and Development Division, Hayashikane Sangyo Co., Ltd., Shimonoseki, Yamaguchi 750-8608, Japan*

[2]*College of Food and Biological Engineering, Jimei University, Xiamen, Fujian 361021, China*

[3]*Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, Nagasaki 852-8521, Japan*

[†] Corresponding author. Fax: +81-95-819-2831. *E-mail address*: t-oda@nagasaki-u.ac.jp (T. Oda)

Abstract

A sulfated polysaccharide ascophyllan inhibited α-glucosidase in a concentration dependent manner, and more than 90% activity was inhibited at 1.0 mg/mL. The inhibitory activity was much higher than that of acarbose. No significant inhibitory effect of ascophyllan on α-amylase was observed up to 10.0 mg/mL. Ascophyllan HS, a commercially available ascophyllan preparation showed even higher inhibitory effect on α-glucosidase than ascophyllan. Interestingly, ascophyllan and ascophyllan HS induced the secretion of glucagon-like peptide-1 (GLP-1) from human intestinal NCI-H716 cell line in a concentration dependent manner (10~100 ng/mL). The oral glucose tolerance tests revealed that after continuous 8-week ingestion of ascophyllan HS at 100 mg/day, the glucose area under the curve values of the ascophyllan HS ingested group were significantly lower than placebo ingested group. Serum glycosylated hemoglobin (HbA1c) level in ascophyllan HS ingested group tended to decrease after 8-week ingestion, whereas no significant change was observed in placebo ingested group. This is the first report indicating that ascophyllan can induce the secretion of GLP-1 from human intestinal cell line (NCI-H716), besides the potent inhibitory effect on α-glucosidase. Furthermore, clinical trial suggested that ascophyllan HS may be a practically applicable blood glucose controlling agent in humans.

Key words: *Ascophyllum nodosum*; ascophyllan; ascophyllan HS; α-glucosidase inhibitor; secretagogue of GLP-1; anti-diabetic effect; HbA1c

1. Introduction

Diabetes mellitus is one of the global human health problems [1]. It is a complex disease defined as a metabolic disorder characterized by elevation of blood glucose levels and is associated with irregular metabolism of various nutrients. This disease is classified as either type 1 or type 2. Type 1 is due to inadequate synthesis of insulin by β-cells of the pancreas, while type 2 is characterized primarily by insulin resistance or the result of insufficient insulin production by β-cells [2]. It is estimated that the number of people with diabetes accounts more than 300 million in the world. Type 2 diabetes is considered as a preventable lifestyle related disease, and dietary control is suggested as a safe and effective nutritional treatment for this disease in addition to usual medical treatments [3-6].

One of the effective therapeutic strategies for type 2 diabetes is to decrease the rate of blood sugar absorption from the small intestine by inhibiting the digestion of dietary starch, the major dietary source of glucose. α-amylase and α-glucosidase are the key enzymes to digest dietary starch into glucose, and the inhibitors of these enzymes have been studied as therapeutic agents to control blood sugar levels [7-9]. Attempts have been made for seeking α-amylase and α-glucosidase inhibitors that can be used as food additives or food supplement. Naturally occurring phenolic compounds are known to show inhibitory effects on these enzymes [10-12]. Seaweeds have been considered as rich source of bioactive substances including enzyme inhibitors. Extracts prepared from algal species contain some polyphenolic compounds with the activity to inhibit α-glucosidase [13-15]. In addition to polyphenolic compounds, polysaccharides isolated from algae have become attractive in the biomedical area because of their numerous bioactivities [16, 17]. In general, polysaccharides derived from marine algae cannot be digested completely by the human digestive system, and therefore they have potentials to act as dietary fiber [18.]. It has been reported that consumption of seaweed fiber can result in a significant

reduction of chronic diseases such as diabetes, obesity, and high blood pressure [19].

Glucagon-like peptide-1(GLP-1) is an incretin hormone that is released by intestinal L cells localized in the distal ileum and colon [20] after nutrient ingestion [21, 22], and it promotes glucose-stimulated insulin secretion by pancreatic β-cells [20]. GLP-1 also reduces glycemia through promoting β-cell proliferation [23], and inhibition of glucagon secretion [24]. Furthermore, GLP-1 has been shown to promote satiety and reduce food intake [25, 26]. Therefore, GLP-1 is a promising therapeutic target for type 2 diabetes, and some of the clinically used anti-diabetic drugs are mimicking or enhancing GLP-1 action [27]. Recent studies are now focusing on discovering of natural compounds which can stimulate intestinal secretion of GLP-1.

Ascophyllum nodosum, a brown alga, is often used as raw material for the preparation of acidic polysaccharide alginate at industrial level and utilized in food consumption. In addition to alginate, *A. nodosum* contains ascophyllan (xylofucoglycuronan) as a sulfated fucan polysaccharide distinguishable from fucoidan, a well-known sulfated polysaccharide [28]. Similar to fucoidan, ascophyllan has various biological activities such as antitumor [29, 30], antioxidant [31], and immune modulating [32-34] activities. Our previous study found that ascophyllan exhibits a growth-promoting activity on MDCK cells, while fucoidan was rather toxic to this cell line [35]. Therefore, ascophyllan is an attractive bioactive polysaccharide with multiple bioactivities for the applications as supplement or pharmaceutical agents. During the courses seeking the new bioactivities and the practical application of ascophyllan, we found that ascophyllan is capable of inhibiting α-glucosidase activity and inducing GLP-1 secretion from human intestinal NCI-H716 cell line. We also found that ascophyllan HS, a commercially available *A. nodosum* preparation, which contains ascophyllan as a main ingredient, alleviated the increase in blood glucose level in clinical trial. In this study, we report the anti-diabetic activities of ascophyllan and ascophyllan HS observed in *in vitro* and *in vivo* systems.

2. Materials and methods

2.1. Materials.

Acarbose, 4-nitrophenyl α-D-glucopyrinoside (PNPG), α-glucosidase, and α-amylase were obtained from Wako Pure Chemical Industries, Ltd., (Osaka, Japan). RPMI 1640 medium, Dulbecco's modified Eagle's medium (DMEM), and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma-Aldrich, Co. (St. Louis, MO, USA). Other chemicals used in the study were of the commercially available highest grade.

2.2. Preparation of ascophyllan HS and ascophyllan.

Brown seaweed *A. nodosum* collected on the coast of Norway was obtained from KAISEI (Shimonoseki, Japan). Ascophyllan HS was prepared by the following procedures. Milled *A. nodosum* was suspended in water and stirred at 90ºC for 45 min. The water extraction was repeated once at 90ºC for 60 min. After filtration, activated charcoal was added to the filtrate and stirred at 90ºC for 30 min. After removal of activated charcoal by filtration, the solution was subjected to spray drying, and the obtained powder was used as ascophyllan HS. Ascophyllan HS is currently commercially available from Hayashikane Sangyo Co. Yamaguchi, Japan. Composition analysis indicated that estimated contents of carbohydrate, protein, lipids, water, and ash in 100 g of ascophyllan HS were 65.8, 0.8, 2.5, 7.4, and 23.5 g, respectively. More than 20 g of purified ascophyllan can be prepared from 100 g of ascophyllan HS by purification procedure reported previously [28]. From the viewpoint of the food safety, acute oral toxicity test using female rat and reverse mutation test using *Echerichia coli* and *Salmonella typhimurium* were conducted on ascophyllan HS. The results indicated that

LD_{50} of ascophyllan HS was over 2000 mg/kg body weight, and the mutagenicity was undetectable. Highly purified ascophyllan was prepared from *A. nodosum* as described previously [28].

2.3. α-glucosidase assay

Inhibitory effect of samples on α-glucosidase was determined by the method reported previously [36]. In brief, 100 µL of α-glucosidase (0.4 U/mL) in 100 mM of phosphate buffer (pH 7.0) was mixed with 100 µL of varying concentrations of each sample in the buffer, and incubated for 10 min at 25°C, and then 250 µL of 2 mM 4-nitrophenyl α-D-glucopyrinoside (PNPG) in the buffer (pH 7.0) was added. After 20 min incubation at 37°C, the absorbance of 4-nitrophenol released from PNPG was measured at 405 nm by a Multiskan GO scanner (Thermo Fisher Scientific Inc., MA, USA). Acarbose, a known inhibitor of α-amylase and α-glucosidase, was used as comparative inhibitor. The inhibition ratios were calculated as follows: % inhibition = $(1\text{-}A_{sample}/A_{control}) \times 100\%$ where A_{sample} is the absorbance in the presence of sample, and $A_{control}$ is the absorbance of reaction mixture without sample.

2.4. α-amylase assay

Inhibitory effect of samples on α-amylase was determined by iodo-starch reaction [11]. In brief, 125 µL of varying concentrations of each sample in 20 mM piperazine-1,4-bis(2-ethanesulfonic acid) buffer (pH 6.9) containing 50 mM NaCl and 5 mM $CaCl_2$ were mixed with 125 µL of α-amylase (final concentration: 15 IU/mL), and incubated at 25°C for 10 min. After the addition of 250 µL of soluble starch (1%, w/v) in above mentioned buffer to the reaction mixture, the mixture was incubated at 37°C for 10 min. To the reaction mixture, 0.5 M acetate/0.5 M HCl solution was added to stop the reaction, and then 1000 µL of iodine solution (0.005% of iodide and 0.05% KI solution)

was added for color development. The absorbance at 660 nm was measured using a Multiskan GO scanner (Thermo Fisher Scientific Inc., MA, USA). The inhibition ratios were calculated as follows: % inhibition = $[1-(A_{blanck}-A_{sample})/(A_{blanck}-A_{control})] \times 100\%$ where A_{sample} is the absorbance in the presence of sample, and $A_{control}$ is the absorbance of reaction mixture without sample, and A_{blanck} is the absorbance of the sample with substrate but no enzyme.

2.5. Cell culture

The human intestinal NCI-H716 cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). The cells in suspension were cultured in a CO_2 (5%) incubator at 37°C in RPMI 1640 minimum supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin, which was used as the growth medium throughout the experiments unless otherwise specified.

2.6. GLP-1 secretion assay

GLP-1 secretion from NCI-H716 cells was conducted as described previously with slight modification [37]. In brief, the cells in the growth medium were harvested by centrifugation (2,000 × *g* for at 4°C for10 min). To prepare the adherent cell monolayer, the pelleted cells were suspended in low glucose DMEM supplemented with 10% FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin, and were seeded into 48-well (2×10^5 cells/well) culture plates coated with Matrigel (Becton Dickinson and Co., Bedford, MA, USA), and cultured at 37°C for 2 days. The medium was replaced with Krebs-Ringer Bicarbonate Buffer (KRB) supplemented with 0.2% (w/v) bovine serum albumin (BSA) containing varying concentrations of each test sample. The cells were then incubated 37°C for 2 h. The cells were also treated with phorbol 12-myristate

13-acetate (PMA) at 100 ng/mL as a positive control. Supernatants were collected with the addition of 50 μg/mL phenylmethylsulfonyl fluoride and frozen at -80°C until use. The levels of active form of GLP-1 in the supernatants were determined by enzyme linked immunosorbent assay (Merck-Millipore) following the manufacturer's instruction.

2.7. Experimental design for human study

A randomized, double-blind experimental study was performed in clinical trial. Ten healthy male and female volunteers (age 22-53 years) were recruited at Hayashikane Sangyo Co., Ltd.. All participants provided written informed consent. In our previous clinical studies, we found that oral ingestion of ascophyllan HS at 100 mg/people for 8 weeks resulted in the significant increase in the blood NK (natural killer) activity and serum interferon-γ level, suggesting that 100 mg/people can be an effective dose to influence the human immune system. More than that, acute oral toxicity tests using female rat indicated that LD_{50} of ascophyllan HS was estimated to be higher than 2,000 mg/kg body weight. Considering these findings and the practical usage of ascophyllan HS in human being, we considered that 100 mg/people is effective and safety dose of ascophyllan HS in this study. Participants were randomly separated to test and control groups, and were requested daily intake of a capsule containing 100 mg ascophyllan HS (test group) or 100 mg glucose (placebo group) after usual dinner meal for 8 weeks. After 0, 4 weeks, and 8 weeks ascophyllan HS ingestion, oral glucose tolerance tests (OGTT) were performed. After overnight fast, the first venous blood sample was taken 30 min before the start of an OGTT for getting 0-time value. After ingestion of 30 g glucose, the blood glucose levels of volunteers were measured at 30, 60, 90, and 120 min by glucose oxidase-dependent colorimetric method using Medi safe mine system (TERUMO Co., Tokyo, Japan).

2.8. Blood glycosylated hemoglobin level

Fresh anticoagulated blood (10 mL) obtained before and after 4 weeks and 8 weeks ascophyllan HS or placebo ingestion was used to measure glycosylated hemoglobin A1c (HbA1c). Blood samples kept at 4°C were sent to the Shimonoseki Medical Association (Yamaguchi, Japan), where HbA1c was quantitatively measured.

2.9. Statistical analysis

The data were expressed as means ± standard errors (SE), and were analyzed by paired Student's t-test to evaluate significant differences. A value of 0.05 was considered statistically significant.

3. Results and discussion

3.1. Inhibitory effects of ascophyllan and ascophyllan HS on α-glucosidase and α-amylase

As shown in Fig. 1, both ascophyllan and ascophyllan HS exhibited inhibitory effect on α-glucosidase activity in a concentration dependent manner. At the concentrations of 0.01 and 0.1 mg/mL, ascophyllan HS showed evidently stronger inhibitory effects than those of ascophyllan, whereas both ascophyllan and ascophyllan HS showed the maximum inhibitory effects at 1.0 and 10.0 mg/mL. These results suggest that ascophyllan HS showed greater α-glucosidase inhibitory activity than ascophyllan at low concentration-range. Fifty% inhibitory concentration (IC_{50}) of ascophyllan and ascophyllan HS was estimated to be 0.50 and 0.05 mg/mL, respectively. These values were much lower than that of acarbose, a clinically used α-glucosidase inhibitor, and the IC_{50} value of acarbose was estimated to be 10.0 mg/mL under the same experimental conditions. To our best knowledge, this is the first report indicating that ascophyllan has

an inhibitory activity on α-glucosidase with even greater activity than acarbose. Ascophyllan HS is a crude polysaccharide product prepared from powdered *A. nodosum* with low-cost performance, and thus may contain beneficial ingredients such as fucoidan and polyphenol compounds other than ascophyllan. Since seaweed-derived polyphenol compounds are known to exhibit α-glucosidase inhibitory activity [38], superior inhibitory activity of ascophyllan HS as compared to ascophyllan may be derived from additive or synergistic effects of multiple beneficial ingredients which can contribute to the inhibition of α-glucosidase. Further studies are needed to clarify this point.

In spite of the potent inhibitory activity of ascophyllan HS and ascophyllan on α-glucosidase, both of them did not show any significant inhibitory effect on α-amylase up to 10.0 mg/mL, whereas nearly 90% of α-amylase activity was inhibited by acarbose at 10.0 mg/mL (Fig. 1). Although the inhibition of α-amylase may also help moderate the release of glucose from starch, its excessive inhibition could provoke intestinal disorders. In fact, it has been reported that acarbose causes intestinal gas production, abdominal distension, and diarrhea as the side effects [39]. The inhibition of α-amylase by acarbose might result in the release of undigested large starch fragments to the lower gastrointestinal tract and subsequent their abnormal fermentation by intestinal microflora [39]. Therefore, it is considered that ascophyllan HS and ascophyllan might not induce the side effects caused by acarbose.

Regarding seaweed-derived polysaccharides with α-glucosidase inhibitory activity, it has been reported that fucoidans isolated from brown algae *Fucus vesiculosus* and *A. nodosum* showed inhibitory activities against α-glucosidase [40]. Comparative studies between fucoidans extracted from *F. vesiculosus* and *A. nodosum* demonstrated that fucoidan from *A. nodosum* showed consistently greater inhibitory effect than fucoidan from *F. vesiculosus*, although the inhibitory activity of these fucoidans significantly differed depending on algal harvesting season [40]. Chemical structural characteristics of fucoidans such as sulfate level, monosaccharide composition, and molecular size vary depending on algal species, the extraction process, and even the

harvest seasons and local climatic conditions [40-45]. The structural analysis suggested that molecular weight and the number of sulfate groups of fucoidan might influence the enzyme inhibitory activity [40]. Similar to fucoidan, ascophyllan is a fucose-containing sulfated polysaccharide discovered from *A. nodosum* as a distinguishable fraction from fucoidan [28]. Our previous studies demonstrated that ascophyllan has a growth-promoting activity on MDCK cells, while fucoidan was rather toxic to this cell line [35]. This finding clearly indicates that there is a difference in the bioactivities of ascophyllan and fucoidan, even though there are some structural similarities between these polysaccharides. Interestingly, fucoidans have been reported to show extremely greater inhibitory activity against α-glucosidase than acarbose [46] as seen in ascophyllan (Fig. 1). Hence, it seems likely that the potent α-glucosidase inhibitory activity is a common feature of sulfated fucose-containing polysaccharides, although further studies are needed to clarify the exact action mechanisms in terms of structure-activity relationship.

3.2. Ascophyllan and ascophyllan HS stimulate GLP-1 secretion in NCI-H716 human intestinal cell line

Glucagon-like peptide-1 (GLP-1) is a peptide hormone consisted of 30 amino acids, that is released from intestinal epithelial L cells after nutrient ingestion [20]. The main physiological role of this endocrine hormone is promoting glucose-dependent insulin secretion from pancreatic β-cells [20]. GLP-1 is also known to have various anti-diabetic effects including promoting β-cells proliferation [23], suppression of glucagon release [24], increase in satiety and reduction of food intake [25, 26]. Therefore, stimulation of GLP-1 secretion from intestinal L cells is an attractive therapeutic option for the management of metabolic syndrome and type 2 diabetes mellitus. For the identification of compounds which stimulate GLP-1 secretion from intestinal L cells, cellular models using human intestinal NCI-H716 cell line and other animal origins have

been reported, and the *in vitro* systems used the cell lines provided useful information regarding naturally occurring compounds with GLP-1 releasing activity [37, 47]. Under these circumstances, we investigated the effect of ascophyllan and ascophyllan HS on NCI-H716 cells in terms of the stimulation of GLP-1 secretion. As shown in Fig. 2, ascophyllan and ascophyllan HS stimulated GLP-1 secretion in NCI-H716 cells in a concentration dependent manner. These results show for the first time that sulfated fucose-containing polysaccharide like ascophyllan is capable of stimulating GLP-1 secretion from human intestinal L cells. It has been reported that protein kinase C (PKC) signaling pathway is involved in the stimulation of GLP-1 secretion, and phorbol 12-myristate 13-acetate (PMA), an activator of PKC stimulates GLP-1 secretion in NCI-H716 cells [37]. Hence, we used PMA as a positive control in this study. Consistent with the previous report [37], PMA at 100 ng/mL induced GLP-1 secretion in NCI-H716 cells at nearly 200% of control level, and almost equivalent stimulating effects of ascophyllan and ascophyllan HS were observed at 100 ng/mL (Fig. 2).

3.3. Suppressible effect of ascophyllan HS on the increase in postprandial blood glucose level in clinical trial

The *in vitro* activities of ascophyllan and ascophyllan HS such as potent inhibition of α-glucosidase and stimulation of GLP-1 secretion from intestinal L cells can contribute to the reduction of postprandial blood glucose level and eventual anti-diabetic effect. To investigate this possibility, we conducted preliminary clinical trial in humans. Considering that ascophyllan HS has already been used as an ingredient of food supplement in Japan, we investigated the effects of ascophyllan HS on the postprandial blood glucose levels in healthy volunteers with average 39.1 years-old. The participants ingested a capsule containing 100 mg of ascophyllan HS or glucose (placebo) once daily after dinner. After 8 weeks, a 30-g oral glucose tolerance tests (OGTT) were conducted. As shown in Fig. 3, the blood glucose levels of ascophyllan HS ingested group tended to

be lower than placebo ingested group at 8-week throughout the measurement times (0~120 min), whereas no significant differences between two groups were observed at initial time and at 4-week. Reflecting the results, the area under the curve (AUC) value of the ascophyllan HS at 8-week was statistically lower than that of placebo ingested group, but not at initial and 4-week time periods. To further investigate the effects of long-term ingestion of ascophyllan HS on blood glucose homeostasis, serum glycosylated hemoglobin (HbA1c) levels were determined. Hemoglobin A1c (HbA1c) is a hemoglobin variant that is formed when glucose binds covalently to hemoglobin molecule *via* non-enzymatic process, and is expressed as the ratio between glycated HbA1 and total HbA1. The binding of the glucose occurs continually during the life span of the erythrocyte and is dependent on blood glucose concentration and the duration of exposure of the erythrocyte to blood glucose [48]. Hence, HbA1c reflects the average plasma glucose concentration the preceding ~90 days depending on the individual [48]. It has been reported that diabetic patients have 2~3 times more HbA1c than healthy individuals, and the cutoff value of >6.0% has a specificity of 100% to detect diabetes in patients on admission [48]. Since the studies were conducted in healthy volunteers, all the HbA1c values obtained were within normal healthy levels (5.0-5.8%). Although no significant differences between ascophyllan HS- and placebo-ingested groups were observed in the actual HbA1c values during the measurement intervals, the degree of fluctuation of HbA1c values in ascophyllan HS group at 8-week compared to the initial level was significantly lower than that of the placebo ingested group (Fig. 4). These results suggest that long-term ingestion of ascophyllan HS may slightly improve the plasma HbA1c value. Further evaluation of the effects of ascophyllan HS on plasma HbA1c values are necessary especially in the patients with diabetes.

4. Conclusion

In *in vitro* analyses, we found that ascophyllan and ascophyllan HS show potent

α-glucosidase inhibitory activity and stimulating activity of GLP-1 secretion from human intestinal L cell line (NCI-H716), suggesting that they can reduce the blood glucose level in two different ways. In oral glucose tolerance test, slight reduction of blood glucose level was observed in ascophyllan HS ingested group (100 mg/day for 8 weeks) as compared to placebo ingested group, which were reflected in the AUC values. Furthermore, slight but statistically significant lowering of serum glycosylated hemoglobin (HbA1c) level was observed in ascophyllan HS ingested groups after 8 weeks as compared to the initial level, whereas no significant change in HA1c level was observed in placebo ingested group. These results suggest that ascophyllan HS has a potential as a promising blood glucose controlling or anti-diabetic agent.

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Figure legends

Fig. 1. Inhibitory effects of ascophyllan (□) and ascophyllan HS (■) on α-glucosidase (A) and α-amylase (B). Acarbose (▢) was used as a known inhibitor. Data represent the average of triplicate measurements and bars indicate the standard errors. Asterisks indicate significant differences between with and without samples ($p < 0.05$).

Fig. 2. Effects of ascophyllan (□) and ascophyllan HS (■), and PMA (▢) on GLP-1 secretion from NCI-H716 cells. Cells were incubated for 2 h with the indicated concentrations of the test samples. The levels of GLP-1 secreted into the medium were measured by ELISA. Data represent the average of triplicate measurements and bars indicate the standard errors. Asterisks indicate significant differences between with and without samples ($p < 0.05$).

Fig. 3. Oral glucose tolerance tests (OGTTs) at 0 (A), 4 weeks (B), and 8 weeks (C) after ascophyllan HS (●) or placebo (○) ingestion. After overnight fasting, blood glucose levels in the subjects of each group were measured at 0, 30, 60, 90, and 120 min after 30 g glucose ingestion. The area under the blood glucose curve (AUC) for each group was calculated (D). The values are the average of measurements of five subjects and the bars indicate the standard errors.

Fig. 4. Serum glycosylated hemoglobin (HbA1c) levels at 0, 4, and 8 weeks after ascophyllan HS (●) or placebo (○) ingestion. After overnight fasting, serum HbA1c levels in the subjects of each group were measured (A). Degrees of fluctuation of serum HbA1c levels at 4 weeks and 8 weeks after ingestion of ascophyllan HS (●) or placebo (○) were calculated by setting the initial value 0 (B). The values are the average of measurements of five subjects and the bars indicate the standard errors. Asterisks indicate significant differences between placebo and ascophyllan HS ($p < 0.05$).

Fig. 1

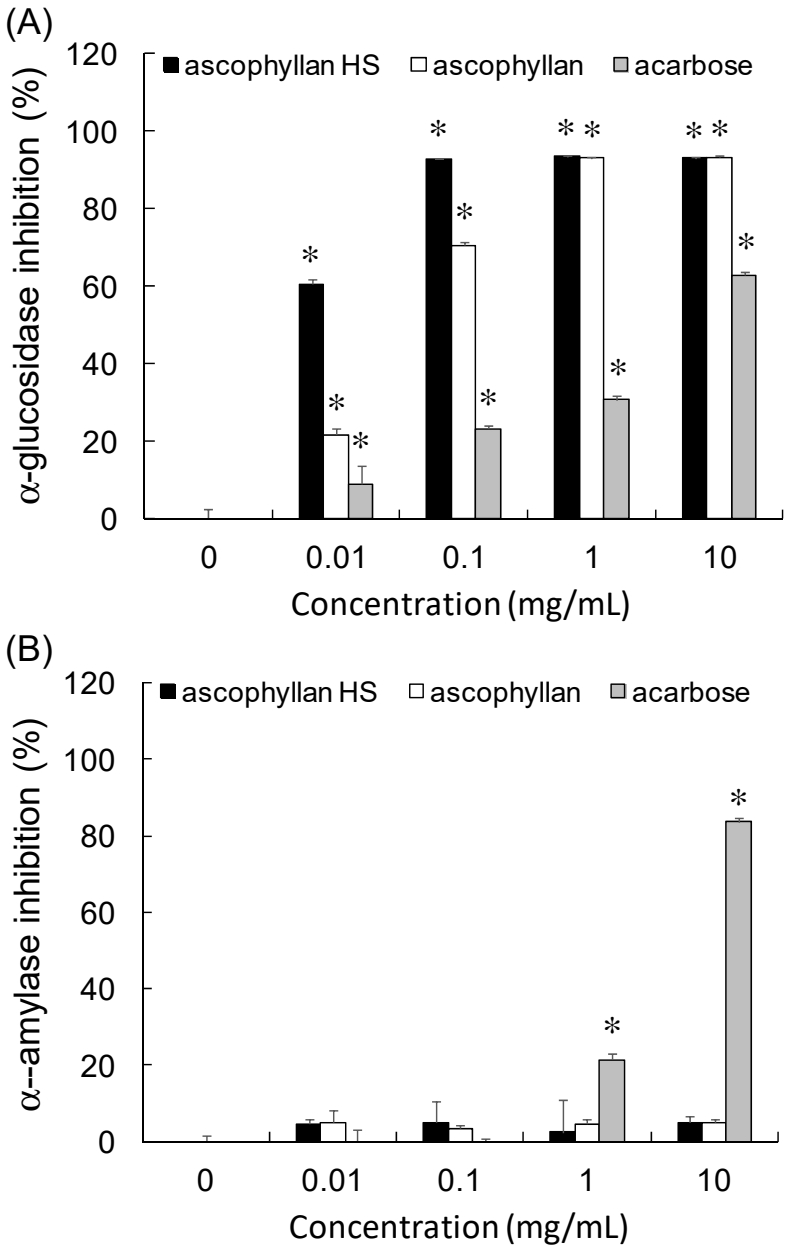

(A)
ascophyllan HS
ascophyllan
acarbose
α-glucosidase inhibition (%)
120
100
80
60
40
20
0
0
0.01
0.1
1
10
Concentration (mg/mL)
(B)
ascophyllan HS
ascophyllan
acarbose
α--amylase inhibition (%)
120
100
80
60
40
20
0
0
0.01
0.1
1
10
Concentration (mg/mL)

Fig. 2

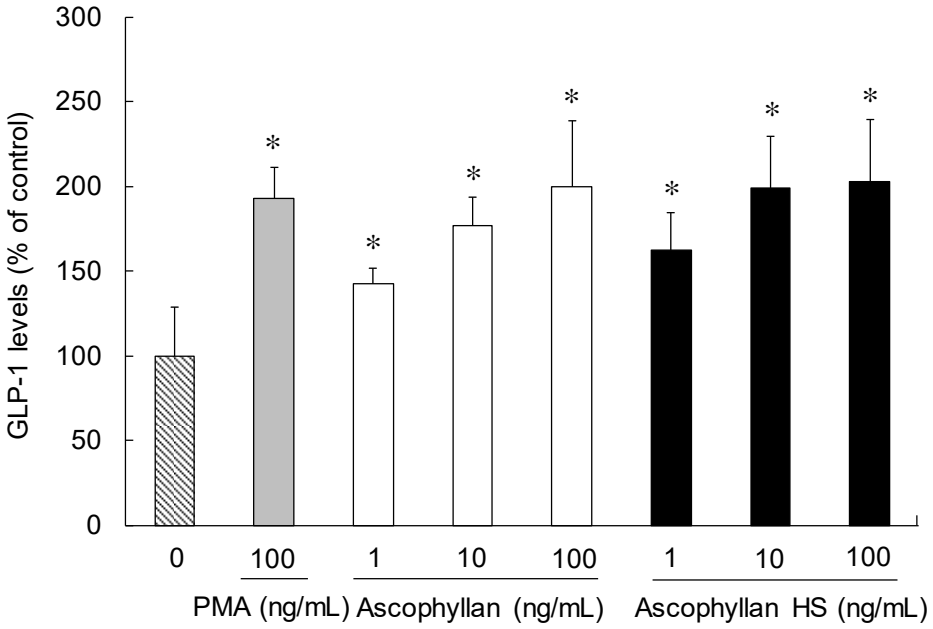

GLP-1 levels (% of control)
300
250
200
150
100
50
0
*
0
100
1
10
100
1
10
100
PMA (ng/mL)
Ascophyllan (ng/mL)
Ascophyllan HS (ng/mL)

Fig. 3

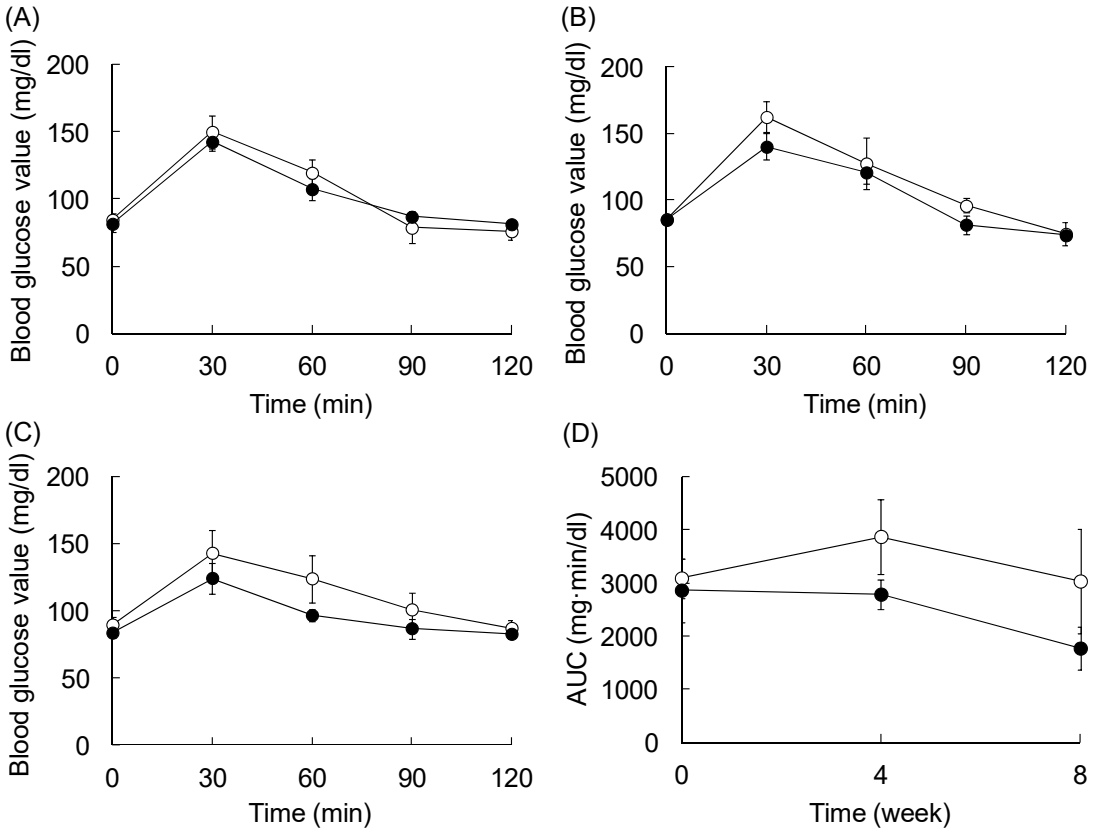
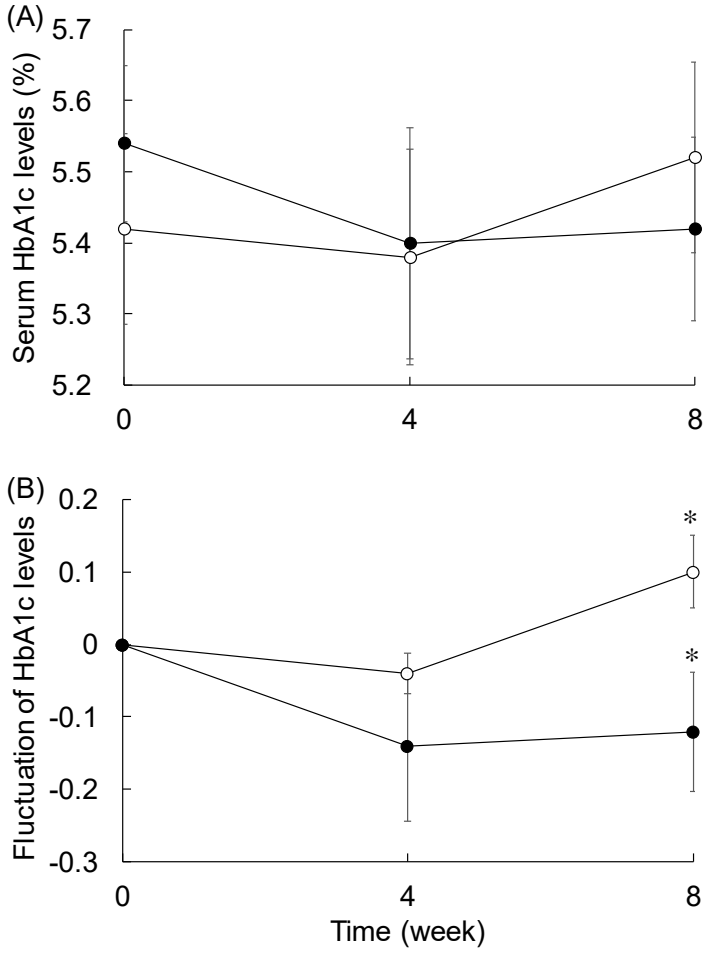

(A)
Blood glucose value (mg/dl)
200
150
100
50
0
0
30
60
90
120
Time (min)
(B)
Blood glucose value (mg/dl)
200
150
100
50
0
0
30
60
90
120
Time (min)
(C)
Blood glucose value (mg/dl)
200
150
100
50
0
0
30
60
90
120
Time (min)
(D)
AUC (mg·min/dl)
5000
4000
3000
2000
1000
0
0
4
8
Time (week)

Fig. 4


(A)
Serum HbA1c levels (%)
5.7
5.6
5.5
5.4
5.3
5.2
0
4
8
(B)
Fluctuation of HbA1c levels
0.2
0.1
0
-0.1
-0.2
-0.3
*
*
0
4
8
Time (week)