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

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1 **Abstract**

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

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

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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

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

2 Glucagon-like peptide-1(GLP-1) is an incretin hormone that is released by
3 intestinal L cells localized in the distal ileum and colon [20] after nutrient ingestion [21,
4 22], and it promotes glucose-stimulated insulin secretion by pancreatic β -cells [20].
5 GLP-1 also reduces glycemia through promoting β -cell proliferation [23], and inhibition
6 of glucagon secretion [24]. Furthermore, GLP-1 has been shown to promote satiety and
7 reduce food intake [25, 26]. Therefore, GLP-1 is a promising therapeutic target for type 2
8 diabetes, and some of the clinically used anti-diabetic drugs are mimicking or enhancing
9 GLP-1 action [27]. Recent studies are now focusing on discovering of natural compounds
10 which can stimulate intestinal secretion of GLP-1.

11 *Ascophyllum nodosum*, a brown alga, is often used as raw material for the
12 preparation of acidic polysaccharide alginate at industrial level and utilized in food
13 consumption. In addition to alginate, *A. nodosum* contains ascophyllan
14 (xylofucoglycuronan) as a sulfated fucan polysaccharide distinguishable from fucoidan, a
15 well-known sulfated polysaccharide [28]. Similar to fucoidan, ascophyllan has various
16 biological activities such as antitumor [29, 30], antioxidant [31], and immune modulating
17 [32-34] activities. Our previous study found that ascophyllan exhibits a
18 growth-promoting activity on MDCK cells, while fucoidan was rather toxic to this cell
19 line [35]. Therefore, ascophyllan is an attractive bioactive polysaccharide with multiple
20 bioactivities for the applications as supplement or pharmaceutical agents. During the
21 courses seeking the new bioactivities and the practical application of ascophyllan, we
22 found that ascophyllan is capable of inhibiting α -glucosidase activity and inducing
23 GLP-1 secretion from human intestinal NCI-H716 cell line. We also found that
24 ascophyllan HS, a commercially available *A. nodosum* preparation, which contains
25 ascophyllan as a main ingredient, alleviated the increase in blood glucose level in clinical
26 trial. In this study, we report the anti-diabetic activities of ascophyllan and ascophyllan
27 HS observed in *in vitro* and *in vivo* systems.

28

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 90°C for 45 min. The water extraction was repeated once at 90°C for 60 min. After filtration, activated charcoal was added to the filtrate and stirred at 90°C 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

1 LD₅₀ of ascophyllan HS was over 2000 mg/kg body weight, and the mutagenicity was
2 undetectable. Highly purified ascophyllan was prepared from *A. nodosum* as described
3 previously [28].

4 5 2.3. *α*-glucosidase assay

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7 Inhibitory effect of samples on *α*-glucosidase was determined by the method
8 reported previously [36]. In brief, 100 μ L of *α*-glucosidase (0.4 U/mL) in 100 mM of
9 phosphate buffer (pH 7.0) was mixed with 100 μ L of varying concentrations of each
10 sample in the buffer, and incubated for 10 min at 25°C, and then 250 μ L of 2 mM
11 4-nitrophenyl *α*-D-glucopyranoside (PNPG) in the buffer (pH 7.0) was added. After 20
12 min incubation at 37°C, the absorbance of 4-nitrophenol released from PNPG was
13 measured at 405 nm by a Multiskan GO scanner (Thermo Fisher Scientific Inc., MA,
14 USA). Acarbose, a known inhibitor of *α*-amylase and *α*-glucosidase, was used as
15 comparative inhibitor. The inhibition ratios were calculated as follows: % inhibition =
16 $(1 - A_{\text{sample}}/A_{\text{control}}) \times 100\%$ where A_{sample} is the absorbance in the presence of sample, and
17 A_{control} is the absorbance of reaction mixture without sample.

18 19 2.4. *α*-amylase assay

20
21 Inhibitory effect of samples on *α*-amylase was determined by iodo-starch
22 reaction [11]. In brief, 125 μ L of varying concentrations of each sample in 20 mM
23 piperazine-1,4-bis(2-ethanesulfonic acid) buffer (pH 6.9) containing 50 mM NaCl and 5
24 mM CaCl₂ were mixed with 125 μ L of *α*-amylase (final concentration: 15 IU/mL), and
25 incubated at 25°C for 10 min. After the addition of 250 μ L of soluble starch (1%, w/v) in
26 above mentioned buffer to the reaction mixture, the mixture was incubated at 37°C for 10
27 min. To the reaction mixture, 0.5 M acetate/0.5 M HCl solution was added to stop the
28 reaction, and then 1000 μ L of iodine solution (0.005% of iodide and 0.05% KI solution)

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

7 8 *2.5. Cell culture*

9
10 The human intestinal NCI-H716 cell line was obtained from the American
11 Type Culture Collection (Manassas, VA, USA). The cells in suspension were cultured in a
12 CO₂ (5%) incubator at 37°C in RPMI 1640 minimum supplemented with 10% fetal
13 bovine serum (FBS), 2 mM L-glutamine, 100 IU/mL penicillin, and 100 µg/mL
14 streptomycin, which was used as the growth medium throughout the experiments unless
15 otherwise specified.

16 17 *2.6. GLP-1 secretion assay*

18
19 GLP-1 secretion from NCI-H716 cells was conducted as described previously
20 with slight modification [37]. In brief, the cells in the growth medium were harvested by
21 centrifugation (2,000 × g for at 4°C for 10 min). To prepare the adherent cell monolayer,
22 the pelleted cells were suspended in low glucose DMEM supplemented with 10% FBS,
23 100 IU/mL penicillin, and 100 µg/mL streptomycin, and were seeded into 48-well (2 ×
24 10⁵ cells/well) culture plates coated with Matrigel (Becton Dickinson and Co., Bedford,
25 MA, USA), and cultured at 37°C for 2 days. The medium was replaced with
26 Krebs-Ringer Bicarbonate Buffer (KRB) supplemented with 0.2% (w/v) bovine serum
27 albumin (BSA) containing varying concentrations of each test sample. The cells were
28 then incubated 37°C for 2 h. The cells were also treated with phorbol 12-myristate

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

5 6 *2.7. Experimental design for human study*

7
8 A randomized, double-blind experimental study was performed in clinical trial.
9 Ten healthy male and female volunteers (age 22-53 years) were recruited at Hayashikane
10 Sangyo Co., Ltd.. All participants provided written informed consent. In our previous
11 clinical studies, we found that oral ingestion of ascophyllan HS at 100 mg/people for 8
12 weeks resulted in the significant increase in the blood NK (natural killer) activity and
13 serum interferon-γ level, suggesting that 100 mg/people can be an effective dose to
14 influence the human immune system. More than that, acute oral toxicity tests using
15 female rat indicated that LD₅₀ of ascophyllan HS was estimated to be higher than 2,000
16 mg/kg body weight. Considering these findings and the practical usage of ascophyllan HS
17 in human being, we considered that 100 mg/people is effective and safety dose of
18 ascophyllan HS in this study. Participants were randomly separated to test and control
19 groups, and were requested daily intake of a capsule containing 100 mg ascophyllan HS
20 (test group) or 100 mg glucose (placebo group) after usual dinner meal for 8 weeks. After
21 0, 4 weeks, and 8 weeks ascophyllan HS ingestion, oral glucose tolerance tests (OGTT)
22 were performed. After overnight fast, the first venous blood sample was taken 30 min
23 before the start of an OGTT for getting 0-time value. After ingestion of 30 g glucose, the
24 blood glucose levels of volunteers were measured at 30, 60, 90, and 120 min by glucose
25 oxidase-dependent colorimetric method using Medi safe mine system (TERUMO Co.,
26 Tokyo, Japan).

27 28 *2.8. Blood glycosylated hemoglobin level*

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

6 7 *2.9. Statistical analysis*

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

12 13 **3. Results and discussion**

14 15 *3.1. Inhibitory effects of ascophyllan and ascophyllan HS on α -glucosidase and* 16 *α -amylase*

17
18 As shown in Fig. 1, both ascophyllan and ascophyllan HS exhibited inhibitory
19 effect on α -glucosidase activity in a concentration dependent manner. At the
20 concentrations of 0.01 and 0.1 mg/mL, ascophyllan HS showed evidently stronger
21 inhibitory effects than those of ascophyllan, whereas both ascophyllan and ascophyllan
22 HS showed the maximum inhibitory effects at 1.0 and 10.0 mg/mL. These results suggest
23 that ascophyllan HS showed greater α -glucosidase inhibitory activity than ascophyllan at
24 low concentration-range. Fifty% inhibitory concentration (IC₅₀) of ascophyllan and
25 ascophyllan HS was estimated to be 0.50 and 0.05 mg/mL, respectively. These values
26 were much lower than that of acarbose, a clinically used α -glucosidase inhibitor, and the
27 IC₅₀ value of acarbose was estimated to be 10.0 mg/mL under the same experimental
28 conditions. To our best knowledge, this is the first report indicating that ascophyllan has

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

9 In spite of the potent inhibitory activity of ascophyllan HS and ascophyllan on
10 α -glucosidase, both of them did not show any significant inhibitory effect on α -amylase
11 up to 10.0 mg/mL, whereas nearly 90% of α -amylase activity was inhibited by acarbose
12 at 10.0 mg/mL (Fig. 1). Although the inhibition of α -amylase may also help moderate the
13 release of glucose from starch, its excessive inhibition could provoke intestinal disorders.
14 In fact, it has been reported that acarbose causes intestinal gas production, abdominal
15 distension, and diarrhea as the side effects [39]. The inhibition of α -amylase by acarbose
16 might result in the release of undigested large starch fragments to the lower
17 gastrointestinal tract and subsequent their abnormal fermentation by intestinal microflora
18 [39]. Therefore, it is considered that ascophyllan HS and ascophyllan might not induce
19 the side effects caused by acarbose.

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

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

15

16 *3.2. Ascophyllan and ascophyllan HS stimulate GLP-1 secretion in NCI-H716 human* 17 *intestinal cell line*

18

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

1 been reported, and the *in vitro* systems used the cell lines provided useful information
2 regarding naturally occurring compounds with GLP-1 releasing activity [37, 47]. Under
3 these circumstances, we investigated the effect of ascophyllan and ascophyllan HS on
4 NCI-H716 cells in terms of the stimulation of GLP-1 secretion. As shown in Fig. 2,
5 ascophyllan and ascophyllan HS stimulated GLP-1 secretion in NCI-H716 cells in a
6 concentration dependent manner. These results show for the first time that sulfated
7 fucose-containing polysaccharide like ascophyllan is capable of stimulating GLP-1
8 secretion from human intestinal L cells. It has been reported that protein kinase C (PKC)
9 signaling pathway is involved in the stimulation of GLP-1 secretion, and phorbol
10 12-myristate 13-acetate (PMA), an activator of PKC stimulates GLP-1 secretion in
11 NCI-H716 cells [37]. Hence, we used PMA as a positive control in this study. Consistent
12 with the previous report [37], PMA at 100 ng/mL induced GLP-1 secretion in NCI-H716
13 cells at nearly 200% of control level, and almost equivalent stimulating effects of
14 ascophyllan and ascophyllan HS were observed at 100 ng/mL (Fig. 2).

15

16 *3.3. Suppressible effect of ascophyllan HS on the increase in postprandial blood glucose* 17 *level in clinical trial*

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

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

25

26 **4. Conclusion**

27

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

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

11

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15

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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 ($p < 0.05$).

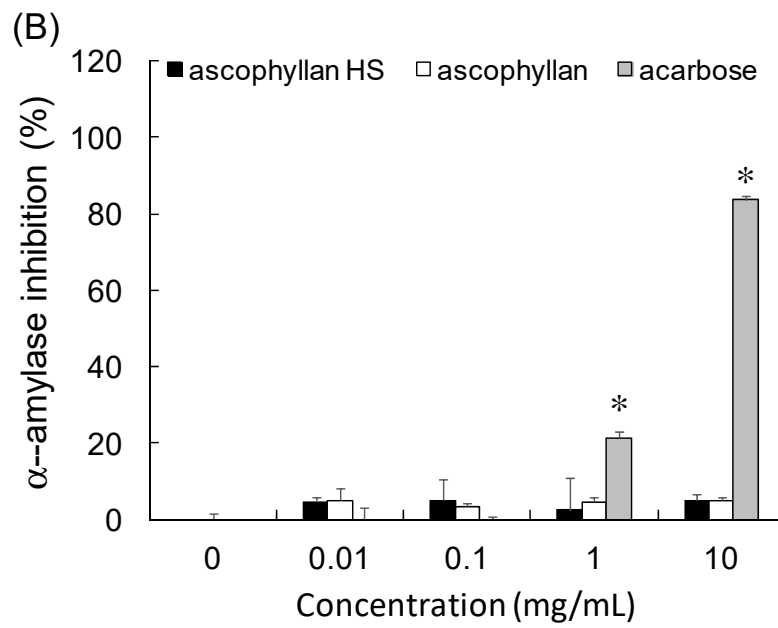
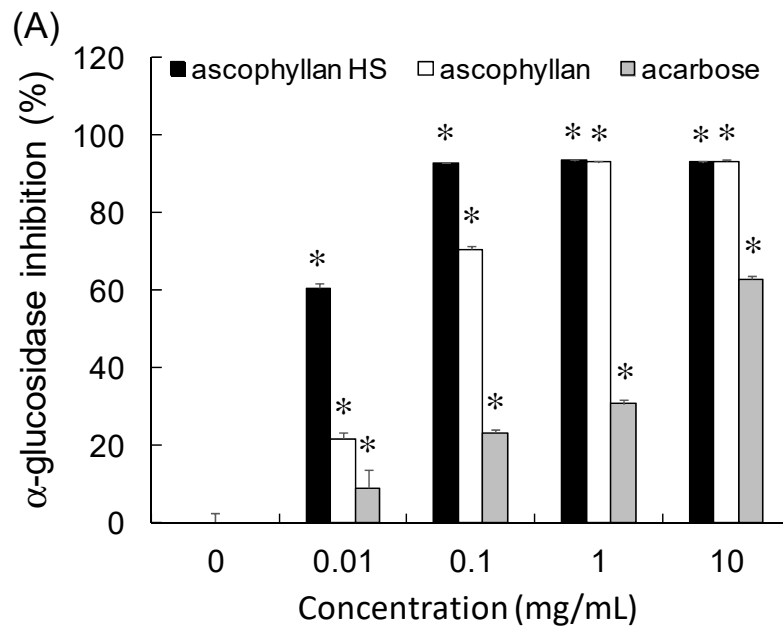
Fig. 3. Oral glucose tolerance tests (OGTTs) at 0 (A), 4 weeks (B), and 8 weeks (C) after ascophyllan HS (●) or placebo (○) ingestion. After overnight fasting, blood glucose levels in the subjects of each group were measured at 0, 30, 60, 90, and 120 min after 30 g glucose ingestion. The area under the 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 ($p < 0.05$).

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Fig. 1

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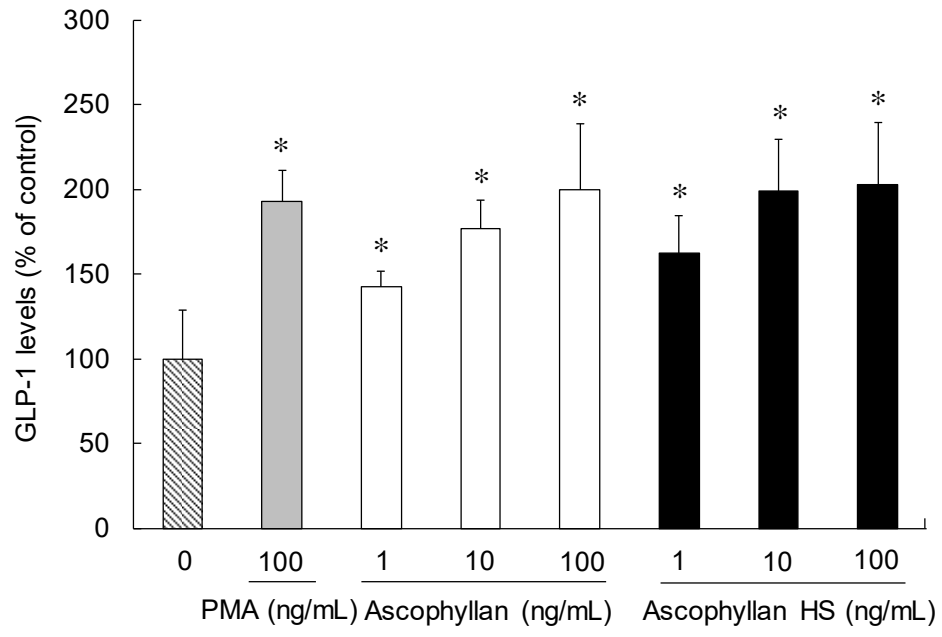
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Fig. 2



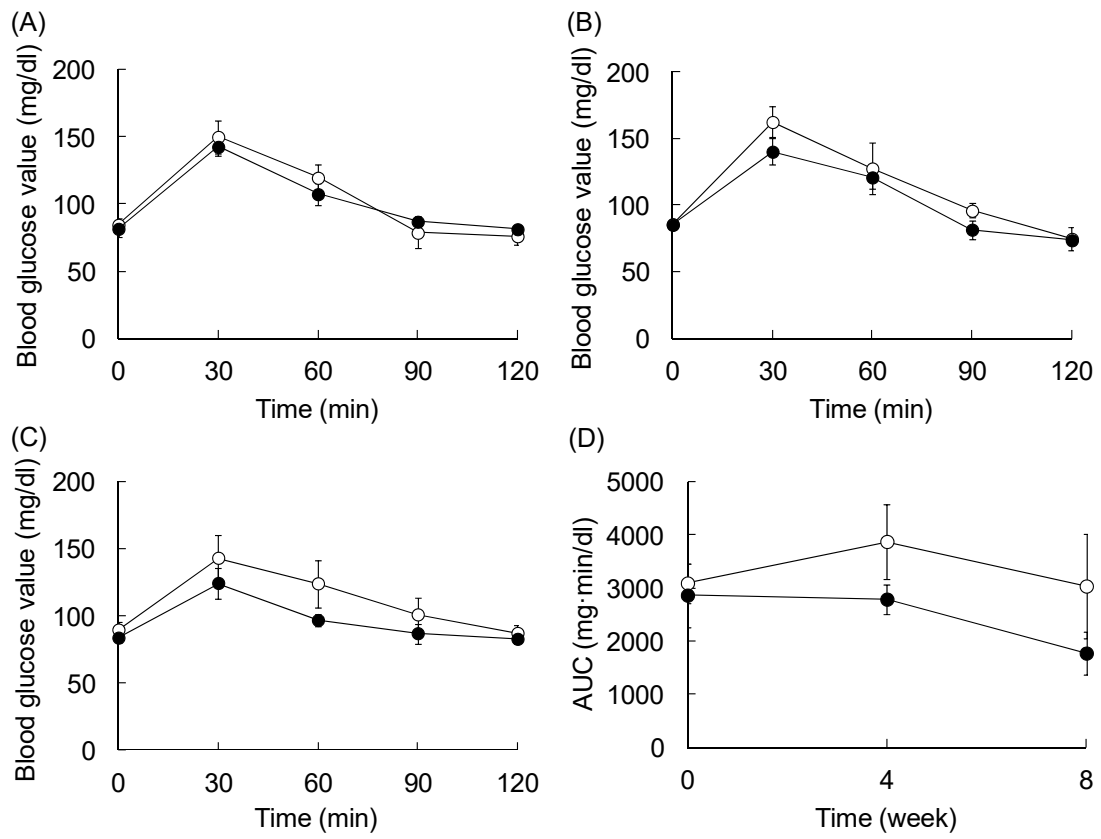
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Fig. 3

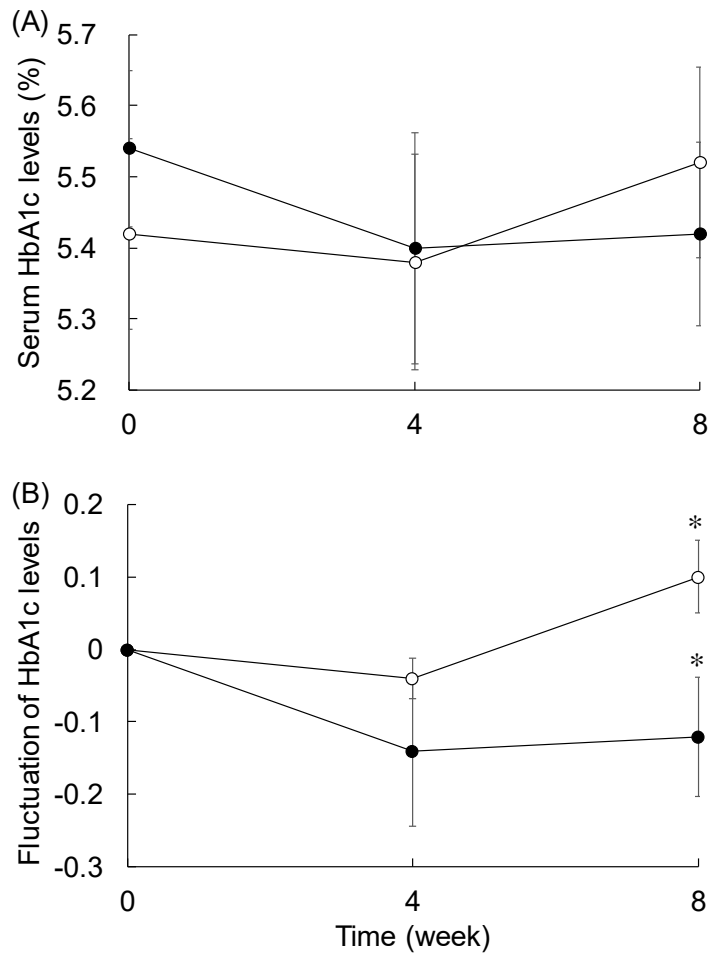


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Fig. 4



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