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Effect of Aqueous Extract from the Root Cortex of *Aralia elata* on Intestinal α-Glucosidases and Postprandial Glycemic Response in Mice

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Abstract

The bark and root cortex of *Aralia elata* have been used as traditional medicine for the treatment of diabetes mellitus in Oriental countries including China, Korea and Japan. The purpose of this study was to assess the influence of aqueous extract from the root cortex of *Aralia elata* on the intestinal α-glucosidase activity and the glycemic response after ingestion of carbohydrates. In *in vitro* intestinal α-glucosidase assay, water extract from the root cortex of *Aralia elata* inhibited the activities of intestinal maltase and trehalase in a concentration-dependent manner with IC_{50} values of 0.45 mg/mL and 0.65 mg/mL, respectively. The fraction which was extracted with water from the root cortex of *Aralia elata* has a stronger inhibitory effect on the intestinal α-glucosidases than that extracted with pure ethanol. In *in vivo* postprandial glycemic response study using mice, the water extract from the root cortex of *Aralia elata* (0.125 g/kg) significantly inhibited the increase in plasma glucose concentration induced by oral administration of maltose and trehalose, but not glucose. The observation indicates that the reduced glucose response to disaccharides in mice with the water extract was, at least in part, due to the inhibition of intestinal α-glucosidase activity. These findings might support the usefulness of a water-based preparation of the root cortex of *Aralia elata*, which has been long used in traditional medicine to treat diabetes mellitus.

Keywords: *Aralia elata*; α-glucosidase; glycemic response; intestine; root cortex; water extract

Introduction

Aralia elata Seem. (Japanese name: Tara-no-ki) (Araliaceae) is an upright deciduous shrub and is native to parts of Eastern Asia including China, Korea and Japan. The bark and root cortex of *Aralia elata* have been used as traditional medicine for the treatment of diabetes mellitus, gastric ulcer and rheumatic arthritis in Oriental countries [1-5]. In addition, the young shoot of the plant, which is called "Tara-no-me" in Japanese, has been used as an edible garnish [6-8].

It has been reported that the methanol extract and saponin fraction from the root cortex of *Aralia elata* exhibited potent hypoglycemic effect on oral sucrose and glucose tolerance tests in rats [9,10]. Among some oleanolic acid glycosides isolated from the root cortex of *Aralia elata*, the oleanolic acid 3-O-monodesmosides elatosides A and E, stipuleanoside R_1 and oleanolic acid 3-O-glycosides showed potent inhibitory activity against the increase in plasma glucose level by oral sucrose tolerance test. In addition, elatosides G, H and I from the young shoot of *Aralia elata* showed hypoglycemic activity in oral sucrose and glucose tolerance tests in rats [6]. Thus, the root cortex and young shoot of *Aralia elata* include various bioactive saponins and glycosides with hypoglycemic activity, which might be associated with anti-diabetic treatment with the plant.

Intestinal α-glucosidase plays an important role in catalyzing the final step in the digestive process of carbohydrates. Inhibition of the carbohydrate-hydrolyzing enzymes such as α-amylase and α-glucosidase in the digestive organs decreases the liberation of glucose from oligosaccharides and delays glucose absorption, resulting in suppression of postprandial plasma glucose levels [11,12]. Recently, various aqueous or alcoholic extracts from various plants have been reported to inhibit intestinal α-glucosidase activities including maltase, sucrase and trehalase [13-19]. The root cortex of *Aralia elata* has been traditionally used in the form of a decoction, which is extracted by boiling in water and concentrated before taken as a medicinal preparation in Japan. However, to our knowledge, it remains to be clarified whether or not the aqueous extract of the root cortex of *Aralia elata* affects the intestinal α-glucosidase activities.

In the present study, we examined the effect of aqueous extract of the root cortex of *Aralia elata* on maltase and trehalase activities in the intestine and the postprandial glycemic response after oral administration of disaccharides such as maltose and trehalose.

Materials and Methods

Materials

The root cortex of *Aralia elata* was purchased from Tochimototenkaido Co. Ltd. (Osaka, Japan). D-glucose, D-maltose monohydrate, D-trehalose dihydrate and horseradish peroxidase were purchased from Sigma-Aldrich (St. Louis, MO, USA). o-Dianisidine was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Glucose oxidase was obtained from Roche (Mannheim, Germany). Acarbose was obtained from Bayer Yakuhin, Ltd. (Osaka, Japan). Voglibose was obtained from Takeda Chemical industries, LTD. (Osaka, Japan). All other chemicals used in the experiments were commercial products of the highest purity available.

Extraction methods

For water extract, five grams of dried root cortex of *Aralia elata* were extracted by boiling for 30 min in 300 mL of distilled water. Then the extracted solution was filtered and freeze-dried. The lyophilized powder was obtained and stored in dark at -20ºC until used for the experiment. For ethanol extract, five grams of dried root cortex of *Aralia elata* were extracted in 100 mL of solvent containing different concentrations of ethanol (30, 70 and 99.5% v/v) for 3 hr at 70ºC. Then the extract was evaporated to dryness, and the powdered extract was stored in dark at -20ºC until used for the experiment. The yields of distilled water, 30%, 70% and 99.5% ethanol extract (w/w) were 22.7, 20.3, 19.4 and 2.6% of the weight of the initial dried root cortex, respectively.

α-Glucosidase assay using moue intestinal mucosa

Experiments with animals were performed in accordance with the Guide for Animal Experimentation, Hiroshima University, and the Committee of Research Facilities for Laboratory Animal Sciences, Hiroshima University. Male ddy mice (10 – 13 weeks old) were deeply anesthetized with diethyl ether. Then, the small intestines were removed and washed with phosphate-buffered saline (PBS buffer containing in mM,137 NaCl, 3 KCl, 8 Na_2HPO_4, 1.5 KH_2PO_4). After the small intestine was excised, the intestinal mucosa was gently scraped off with a glass cover slip on ice. The collected mucosa was centrifuged at 3,000 rpm for 10 min at 4ºC, and was homogenized in an appropriate volume of a buffer containing 50 mM mannitol and 2 mM Tris-Hepes (pH7.1). The homogenate was centrifuged at 3,000 rpm for 10 min at 4ºC, and the resulting supernatant was used for the assay of the activities of maltase and trehalase. A reaction mixture consisting of 300 μL of 20 mM Tris-Hepes buffer (pH7.5) including root cortex extract and 30 μL of intestinal mucosa preparations was preincubated for 5 min at 37ºC. The reaction was initiated by addition of 30 μL of a test substrate solution (final concentration of maltose and trehalose; 5 mM) and performed for 30 min at 37ºC. Then, the glucose concentration of the reaction mixture was determined by the glucose oxidase-peroxidase method with the use of o-dianisidine as the dye [20]. Specific enzyme activity was calculated as micromoles of substrate hydrolyzed per milligram protein within 1 min. Protein concentration was measured by the method of Lowry *et al.* [21] with bovine serum albumin as a standard.

Plasma glucose concentrations after carbohydrate loading in mice

Male ddy mice (7 – 12 weeks old) fasted overnight were employed for sugar loading tests. Control group was orally administered via gavage with disaccharide (maltose or trehalose, 1 g/kg) or glucose (2 g/kg). *Aralia elata* water extract-treated group was orally coadministered via gavage with disaccharide (maltose or trehalose, 1 g/kg) or glucose (2 g/kg) and the water extract (125 mg/kg). The blood samples were taken from the tail vein before (0 min) and 30, 60 and 120 min after loading, and then the blood glucose levels were measured using a simple blood glucose level analyzer GLUTESTACE (Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan).

Data analysis

The half-maximal inhibitory concentration (IC_{50}) values were determined by fitting data with a Hill equation using the KaleidaGraph™ program (Version 3.08, Synergy Software, PA, USA). Statistically significant differences were determined by Student's *t*-test, or one way analysis of variance (ANOVA) with the Tukey-Kramer's test for post hoc analysis. A *P* value less than 0.05 was considered statistically significant.

Results

Effects of acarbose and voglibose, α-glucosidase inhibitors, on maltase and trehalase activities of mouse intestinal mucosa

In order to confirm the activities of α-glucosidase in mouse intestinal mucosa employed in this study, we first examined the effects of acarbose and voglibose, two α-glucosidase inhibitors used in clinical pharmacotherapy, on the hydrolysis of maltose and trehalose in mouse intestinal mucosa. As shown in Fig. 1, the hydrolysis of maltose, reflecting the activity of maltase, in mouse intestinal mucosa was inhibited by acarbose and voglibose in a concentration-dependent manner with the IC_{50} values of 0.40 μg/mL and 0.12 μg/mL, respectively. These IC_{50} values for maltase were comparable to those reported previously in the intestine [13,15]. On the other hand, neither acarbose nor voglibose had a significant effect on the activity of trehalase in mouse intestinal mucosa. Weak or no inhibitory effects of the α-glucosidase inhibitors on trehalase are consistent with previous observations in human and rat intestine [11,22]. Thus, these

results indicated that the activities of intestinal maltase and trehalase could be appropriately evaluated in this study.

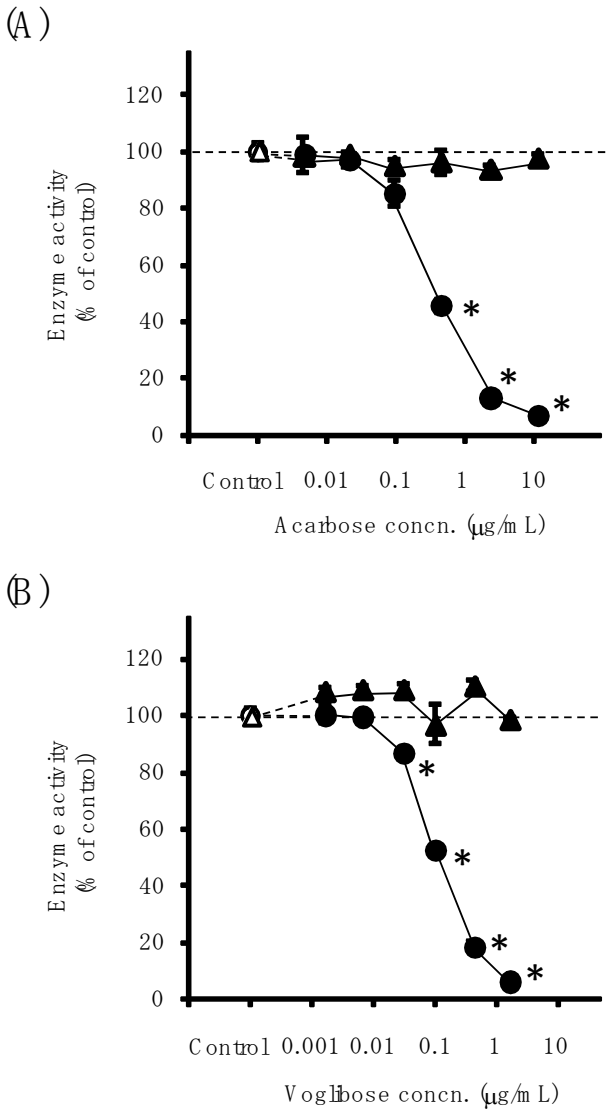

Figure 1. Effects of acarbose and voglibose, α-glucosidase inhibitors, on maltase and trehalase activities of mouse intestinal mucosa. A reaction mixture containing acarbose (A) or voglibose (B) at various concentrations and intestinal mucosa preparations were preincubated for 5 min at 37°C. The reaction was initiated by addition of maltose (circles) or trehalose (triangles) (final concentration 5 mM) and performed for 30 min at 37°C. Then, the concentration of glucose in the reaction mixture was determined by the glucose oxidase-peroxidase method as described in Materials and Methods. Enzyme activities were expressed as percentages of control. Each symbol represents the mean ± S.E. of three determinations. *$p < 0.05$, significantly different from the control.

Concentration-dependent effects of *Aralia elata* water extract on α-glucosidase activities of mouse intestinal mucosa

The root cortex of *Aralia elata* has been traditionally used in the form of a decoction, including various constituents which are extracted from the plant material by boiling in water. Therefore, we examined the effect of water extract from the root cortex of *Aralia elata* on intestinal α-glucosidase activities (Fig. 2). The water extract from the root cortex of *Aralia elata* inhibited the activity of maltase in mouse intestinal mucosa in a concentration-dependent manner with an IC_{50} value of 0.45 mg/mL. In addition, the water extract from the root cortex of *Aralia elata* decreased the intestinal trehalase activity in a concentration-dependent manner with an IC_{50} value of 0.65 mg/mL. This inhibitory effect of the water extract on trehalase was different from those of acarbose and voglibose shown in Fig. 1.

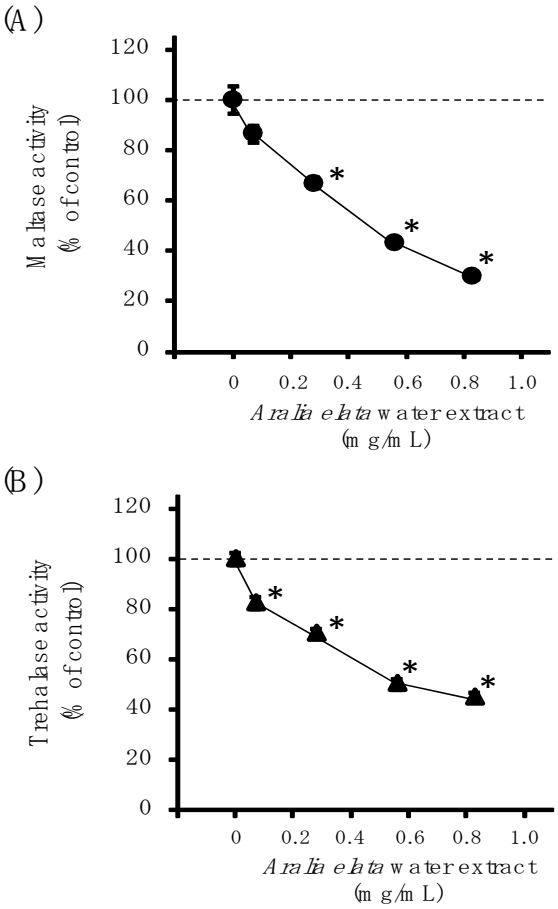

Figure 2. Concentration-dependent effect of *Aralia elata* water extract on -glucosidase activities of mouse intestinal mucosa.
A reaction mixture containing water extract from the root cortex of *Aralia elata* at various concentrations, and intestinal mucosa preparations were preincubated for 5 min at 37°C. The reaction was initiated by addition of maltose (A) or trehalose (B) (final concentration 5 mM) and performed for 30 min at 37°C. Enzyme activities were expressed as percentages of control. Each symbol represents the mean ± S.E. of three determinations. *$p < 0.05$, significantly different from the control. †$p < 0.05$, significantly different from the value of the water extract

Effects of *Aralia elata* extracts extracted with different ethanol concentrations on α-glucosidase activities of mouse intestinal mucosa

The effects of *Aralia elata* extracts from the root cortex extracted with solutions containing different concentrations of ethanol (30%, 70% and 99.5%) on intestinal maltase and trehalase activities were examined, and were compared with those of the water extract (Fig. 3). When each extract was examined at a concentration of 0.56 mg/mL, the inhibitory effects of 30% and 70% ethanol extracts on the intestinal maltase and trehalase activities were observed, and were comparable to the inhibitory effects of the water extract. On the other hand, the pure ethanol (99.5%) extract had significantly less inhibitory potency for maltase and trehalase than the water extract.

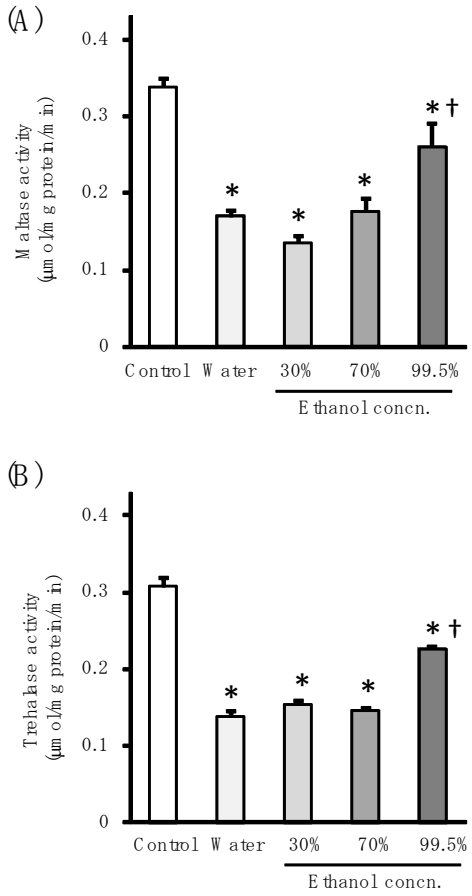

Figure 3. Effects of *Aralia elata* extracts extracted with different ethanol concentrations on -glucosidase activities of mouse intestinal mucosa. A reaction mixture containing extracts from the root cortex of *Aralia elata* extracted with water or different ethanol concentrations (30, 70, 99.5%), and intestinal mucosa preparations were preincubated for 5 min at 37°C. The reaction was initiated by addition of maltose (A) or trehalose (B) (final concentration 5 mM)) and performed for 30 min at 37°C. Enzyme activity was calculated as micromoles of substrate hydrolyzed per milligram protein within 1 min. Each column represents the mean ± S.E. of three determinations. *$p < 0.05$, significantly different from the control.

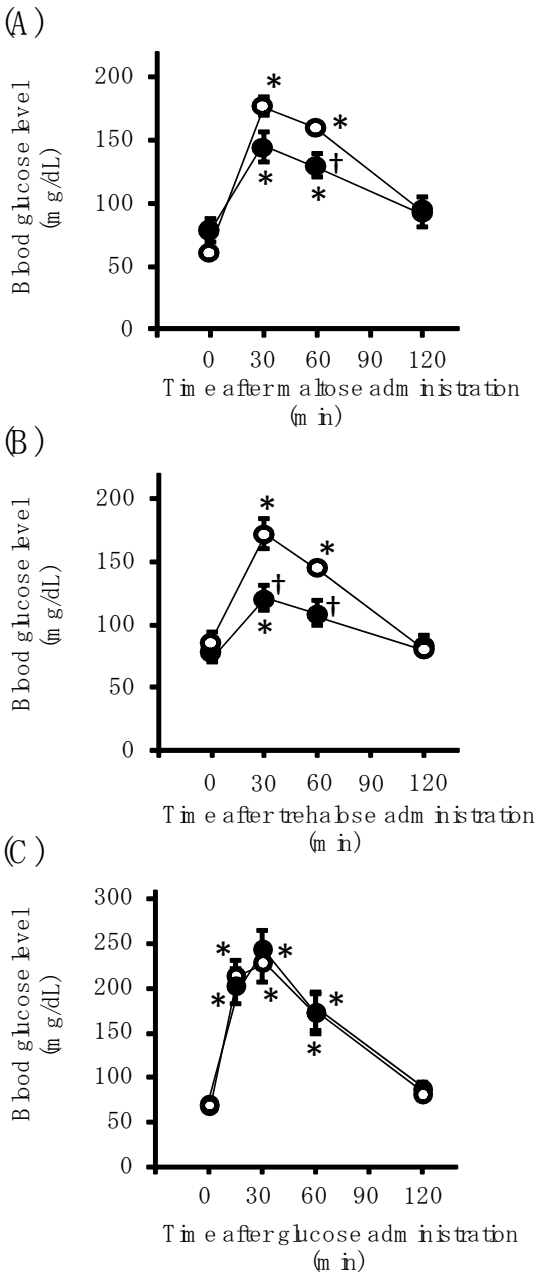

Figure 4. Effect of *Aralia elata* water extract on blood glucose levels in carbohydrate-loaded mice. Male ddy mice fasted overnight was orally administered via gavage with 1g/kg maltose (A), 1 g/kg trehalose (B), or 2 g/kg glucose (C) without (open circles) or with (closed circles) 125 mg/kg water extract of the root cortex of *Aralia elata*. The blood samples were taken from the tail vein before (0 min) and 30, 60 and 120 min after loading. Each symbol represents the mean ± S.E. of four to ten mice. *$p < 0.05$, significantly different from the value before carbohydrate loading (time 0). †$p < 0.05$, significantly different from the value without *Aralia elata* water extract at the same time after carbohydrate loading.

Effect of *Aralia elata* water extract on blood glucose levels in carbohydrate-loaded mice

The effect of *Aralia elata* water extract on the blood glucose changes was investigated in mice orally given maltose, trehalose or glucose (Fig. 4). *Aralia elata* water extract in a dose of 125 mg/kg significantly suppressed the increase in the blood glucose levels 30 to 60 min after maltose or trehalose administration. In contrast, there were no significant changes in the blood glucose levels after loading of glucose between mice without and with *Aralia elata* water extract.

Discussion

Postprandial hyperglycemia is a major risk factor for vascular complications associated with diabetes mellitus. Therefore, controlling postprandial plasma glucose level is critical in the early treatment of diabetes mellitus and in preventing chronic vascular complications [23]. Hyperglycemia, which is characterized by a rapid rise in blood glucose levels, is due to absorption of glucose in the intestine, following hydrolysis of starch by pancreatic α-amylase and α-glucosidases. Therefore, inhibition of intestinal α-glucosidases suppresses the postprandial glycemic increase by delaying carbohydrate hydrolysis. In this study, we examined the effects of aqueous extract from the root cortex of *Aralia elata* on intestinal α-glucosidases and postprandial glycemic response in normal mice.

The water extract from the root cortex of *Aralia elata* inhibited the activities of maltase and trehalase in mouse intestinal mucosa in a concentration-dependent manner with IC_{50} values of 0.45 mg/mL and 0.65 mg/mL, respectively. Recently, a diterpenoid nimbidiol, isolated from the root bark of *Azadirachta indica*, was reported to more potently inhibit intestinal maltase and trehalase than acarbose and voglibose [24]. There are several reports on inhibitory effects of water extract from other plants on intestinal maltase activity. The aqueous extracts from flower buds of *Cleistocalyx operculatus* and guava leaves inhibited the intestinal maltase activity with IC_{50} values of 0.70 mg/mL and 0.97 mg/mL, respectively [14]. In addition, the IC_{50} value of water extract from flowers of *Matricaria chamomilla* for maltase in rat small intestine was reported to be 2.6 mg/mL [15]. Furthermore, the inhibitory effect of aqueous extract from brown alga *Ecklonia stolonifera* on maltase activity in rat small intestine had an IC_{50} value of 4.2 mg/mL [16]. Thus, it is likely that the water extract from the root cortex of *Aralia elata* more potently decreases the intestinal maltase activity, compared to the water extracts from these other plants.

Yoshikawa *et al.*[9,10] found that the glycoside fractions from the root cortex of *Aralia elata* reduced the increase in plasma glucose level in oral sucrose tolerance test. The glycoside fraction including the oleanolic acid glycosides was obtained from the methanolic extract from the root cortex of *Aralia elata*. The glycoside fraction exhibited the hypoglycemic effect on oral sucrose tolerance test in rats [10]. Furthermore, it was shown that some oleanolic acid glycosides, including elatoside A and F, from the root cortex of *Aralia elata* had an inhibitory potency against the rise of plasma glucose level after sucrose loading. Such oleanolic acid glycosides might be, at least in part, involved in the inhibitory potency of *Aralia elata* root water extract on *in vitro* intestinal α-glucosidases and *in vivo* postprandial glycemic response observed in this study. Further studies are needed to characterize the components which inhibit intestinal α-glucosidases in the water extract from the root cortex of *Aralia elata*.

In *in vivo* study, we observed that *Aralia elata* water extract significantly suppressed the increase in the blood glucose levels after oral loading of disaccharides (maltose and trehalose), but not glucose, a form of monosaccharide. Therefore, it is likely that the effect of *Aralia elata* water extract on postprandial glycemic response is due to a decrease in intestinal α-glycosidase activity rather than the membrane transport process of glucose across the intestinal epithelial cells. In contrast with this study, Yoshikwa *et al.* [6] reported that the glucose-loaded elevation of plasma glucose was suppressed by oral administration of the saponin fraction and elatosides G, H and I from the young shoot of *Aralia elata*. Therefore, such bioactive components from the young shoot, which inhibit the intestinal absorption of glucose given orally, might not be included in the water extract from the root cortex of *Aralia elata*.

The yield of the water extract of root cortex of *Aralia elata* amounted to 22.7% of the weight of dried root cortex of the plant. The root cortex of *Aralia elata* is usually taken as a decoction, which is often prepared by boiling 20 g of dried root cortex of *Aralia elata* in 600 mL water until about two-thirds (ca. 400 mL) of the water volume remains [5]. When the yield of the water extract in the decoction is the same as that obtained in this study, the decoction includes the water extract of about 11 mg/mL, which is approximately 24-fold and 17-fold higher than the IC_{50} values for mouse intestinal maltase (0.45 mg/mL) and trehalase (0.65 mg/mL), respectively. Therefore, when the decoction is orally administered, the water extract concentration in the decoction might be high enough to reduce the postprandial glycemic response, resulting from the inhibition of α-glucosidase activity in the intestine.

Conclusion

In conclusion, we found that the aqueous extract from the root cortex of *Aralia elata* exhibited a significant inhibitory potency against intestinal α-glucosidase and the postprandial glycemic response after oral disaccharide loading. These findings might, at least in part, support the usefulness of water-based preparation of the root cortex of *Aralia elata*, which has been traditionally used to treat diabetes mellitus.

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