Phytopharmacology

Inforesights Publishing

# Inhibitory effects of aqueous extract prepared from joint part of lotus root on α-amylase and α-glucosidase activities

Naoyoshi Nishibori[1], Manami Sawaguchi[2], Takara Hiroi[2], Takefumi Sagara[1], Mari Itoh[2], Song Her[3], Mi-Sook Lee[3], Kyoji Morita[2,*]

[1]Laboratory of Cell Biology and Toxicology, Department of Food Science and Nutrition, Shikoku Junior College, Ohjin, Tokushima 771-1192, Japan.
[2]Laboratory of Neuropharmacology, Department of Nursing, Shikoku University School of Health Sciences, Ohjin, Tokushima 771-1192, Japan.
[3]Division of Bio-Imaging, Chuncheon Center, Korea Basic Science Institute, Chuncheon, 200-701, Republic of Korea.

*Corresponding Author: kmorita@shikoku-u.ac.jp

**Received**: 4 March 2012, **Revised**: 15 March 2012, **Accepted**: 16 March 2012

**Abstract**

Postprandial hyperglycemia is a risk factor contributing to cardiovascular complications in type 2 diabetes, and α-amylase and α-glucosidase, the key enzymes in the digestion and absorption of carbohydrates, are recognized to be responsible for the postprandial elevation of blood glucose levels, and the inhibition of these enzymes is therefore considered to be effective to prevent the emergence of postprandial hyperglycemia. Then, the aqueous extracts were prepared from both edible and joint parts of lotus root (a rhizome of *Nelumbo nucifera*), and the inhibitory effects of these extracts on α-amylase and α-glucosidase activities were examined. Consequently, the joint part extract, but not the edible part extract, was shown to inhibit α-amylase and α-glucosidase in a different manner. Therefore, it seems conceivable that the aqueous extract prepared from the joint part of lotus root inhibits both α-amylase and α-glucosidase, thereby effectively preventing the postprandial elevate-on of blood glucose levels in diabetic patients.

**Keywords**: Lotus root; *Nelumbo nucifera;* α-Amylase; α-Glucosidase

## Introduction

Postprandial hyperglycemia is generally known as one of the major risk factors contributing to the development of cardiovascular complications in type 2 diabetes, and therefore necessary to be properly treated at an early stage of the disease for avoiding the tragic terminal conditions of diabetic patients brought by the cardiovascular complications. On the other hand, α-amylase and α-glucosidase are considered as the key enzymes respons-

 

ible for the postprandial elevation of blood glucose levels, and the inhibition of these enzymes is therefore expected to be effective for preventing the emergence of postprandial hyperglycemia (Ceriello, 2005). In the mammalian digestive systems, α-amylase is the enzyme catalyzing the hydrolysis of α-1,4-glycosidic bond of starch and glycogen to oligosaccharides at the first step of the carbohydrate digestion process. Subsequently, oligosaccharides are hydrolyzed by α-glucosidase localized on the brush-border membrane of the small intestine to convert to monosaccharides, which can be transported from the lumen into the blood vessels across the wall of the small intestine. Therefore, it seems quite possible that the postprandial elevation of blood glucose levels can be effectively prevented by inhibiting the transports of monosaccharides from the digestive tract to the circulation system. Based on this concept, the inhibitors of α-amylase and α-glucosidase are developed as the oral anti-diabetic drug for preventing the postprandial hyperglycemia through their inhibitory effects on the digestion and absorption of carbohydrates in patients with type 2 diabetes.

The inhibition of key enzymes involved in the digestion and absorption of carbohydrates has previously been proposed to prevent the postprandial hyperglycemia, and also considered as an important strategy for managing efficiently the blood glucose levels in type 2 diabetic patients (Chiasson, et al., 1998). According to this strategy, it is necessary for diabetic patients to daily intake these enzyme inhibitors without interruption, and therefore seems most convenient and reliable to intake these inhibitors as the supplementary ingredients of the daily meals. For this purpose, α-amylase and α-glucosidase inhibitors from plant sources are currently watched with great interest as the oral effective agents in the prevention of obesity as well as diabetes. In fact, many plant-based medicines and functional foods modulating the digestion and absorption of carbohydrates have been developed until today (Tundis, et al., 2010). For instance, the active substances have been extracted from a variety of leaves, weeds, herbs, berries, nuts and fruits, and the inhibitory effects of these plant extracts on both α-amylase and α-glucosidase have been extensively investigated (Al-Zuhair, et al., 2010; Hogan, et al., 2010; Jo, et al., 2011; Kim, et al., 2000; Subramanian, et al., 2008; Tadera, et al., 2006; Tsujita and Takaku, 2008; Tsujita, et al., 2008). However, although these plant extracts have been shown to cause the inhibitory effects on these enzymes, the active ingredients contained in most of them have not yet been isolated, and still remain unidentified.

Lots of time and efforts have previously been spent to obtain the plant extracts applicable to the postprandial hyperglycemia, but it seems still not enough, and furthermore necessary to find out other plant extracts containing potentially active substances for managing effectively the postprandial elevation of blood glucose levels in diabetic patients. On the other hand, lotus root (a rhizome of *Nelumbo nucifera*) is one of the popular medicinal plants having a variety of pharmacological activities, such as hypolipemic, antiviral, antipyretic, antiinflammatory and antioxidative effects, thereby traditionally employing as a folk medicine in the treatment of diarrhea, gastritis, insomnia and nervous prostration in many countries of East Asia (Jung, et al., 2003; Kuo, et al., 2005; la Cour, et al., 1995; Liu, et al., 2007; Sinha, et al., 2000). Furthermore, the lotus root extract has been analyzed, and shown to contain many phytochemicals including isoliensinine, kaempferol and procyanidins as the major active components (Kim, et al., 2007; Ling, et al., 2005). Moreover, lotus root has been shown to cause hypoglycemic, antidiarrheal and immunomodulatory effects (Mukher-

 

jee, et al., 2010; Mukherjee, et al., 1997; Talukder and Nessa, 1998), and also suggested to have a potential activity improving the functions of learning and memory as well (Yang, et al., 2008). Based on these previous findings, we focused on phytochemicals contained in lotus root, particularly in the joint part, which is the inedible part connecting the edible parts each other and usually dumped as an agricultural waste. In a series of our current studies, the aqueous extracts were prepared from the edible and joint parts of lotus root, and the biological activities of these extracts were then investigated to effectively utilize lotus root as a potential source of bioactive substances. For this purpose, the direct effects of the aqueous extracts prepared from lotus root on both α-amylase and α-glucosidase activities were examined *in vitro* to assess their potential bioactivities to prevent the postprandial elevation of blood glucose levels in diabetic patients.

## Material and methods

### *Chemicals*

Porcine pancreas α-amylase and *p*-nitrophenyl-α-D-glucopyranoside were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Yeast α-glucosidase was purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan). Soluble starch was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals used were commercially available reagent grade.

### *Preparation of lotus root extracts*

Lotus root (a rhizome of *Nelumbo nucifera*) was kindly donated by the Naruto Agricultural Cooperative in Tokushima (Naruto, Japan). To prepare the aqueous extract, lotus root was first cleaned by washing with water, and the edible and joint parts were then sliced in approximately 1 cm thickness. The slices were dehydrated by exposing them to wind stream for several weeks, and then ground into coarse powder. The powder was soaked in distilled water in the ratio of 4 g to 100 ml, and kept it at 4$^{o}$C for 1 h. Then, the suspension was filtered through a Whatman No. 1 filter paper, and the obtained filtrate was then centrifuged at 6,000 x g for 20 min to remove the insoluble materials. The supernatant fraction was further centrifuged at 12,000 x g for 20 min to clarify the solution, and the extract was sterilized by filtering through a 0.2 μm syringe-top disk filter. The obtained extract was aliquoted, and stored at -20 $^{o}$C until use.

### *Determination of α-amylase activity*

The enzyme activity was measured by the method reported previously (Xiao, et al., 2006). Briefly, the sample solution (20 μl) was added to 100 μl of the enzyme solution (0.25 U of the enzyme in 1 ml of the buffer solution consisting of 50 mM Tris-HCl, 20 mM NaCl and 2 mM $CaCl_2$, pH 7.0), and then mixed with 100 μl of the substrate solution (0.5 mg of starch in 50 mM Tris-HCl, pH 7.0) on ice. A pair of the mixtures was prepared, and one of them was incubated at 37$^{\circ}$C for 20 min, and the reaction was stopped by adding 50 μl of 1 M HCl. The other tube was mixed with 50 μl of 1 M HCl, and incubated as a blank. These solutions were mixed with 250 μl of 5 mM iodine solution, and diluted with 750 μl of

 

distilled water. The absorbance at 580 nm was measured, and the enzyme activity was obtained as the difference between these two values.

***Determination of α-glucosidase activity***

The enzyme activity was determined according to the method described previously (Kim, et al., 2000). The sample solution (20 μl) was mixed with 100 μl of the enzyme solution (10 mU of the enzyme in 1 ml of 100 mM phosphate buffer, pH7.0), and preincubated at 37 $^{o}$C for 5 min. The mixture was chilled on ice, and then mixed with 100 μl of the substrate solution (0.5 mM *p*-nitrophenyl-α-D-glucopyranoside in 100 mM phosphate buffer, pH7.0). The complete reaction mixture was incubated at 37 $^{o}$C for 20 min, and the reaction was stopped by adding 100 μl of 1 M $Na_2CO_3$. The mixture was diluted with 400 μl of distilled water, and the absorbance at 405 nm was then determined.

***Statistical analysis***

Results were presented as the mean ± SEM, and the data were analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The difference between two values with $p < 0.05$ was regarded as indicating a statistically significant.

**Results and discussion**

Both α-amylase and α-glucosidase are known as the key enzymes involved in the digestion and absorption of carbohydrates, and considered to be closely associated with the postprandial elevation of blood glucose levels in patients with type 2 diabetes. Therefore, the inhibitions of these enzymes are considered to effectively reduce the risk of cardiovascular complications as a consequence of preventing the postprandial hyperglycemia in diabetic patients. In the present study, the aqueous extracts were prepared from the edible and joint parts of lotus root, and the effects of these extracts on the activities of α-amylase and α-glucosidase were then examined. As shown in Figure 1, the aqueous extract prepared from the edible part of lotus root failed to cause any significant alterations in α-amylase and α-glucosidase activities. In contrast, the joint part extract inhibited both α-amylase and α-glucosidase in a concentration-dependent manner, and caused approximately 95% and 40% reductions of α-amylase and α-glucosidase activities, respectively (Figure 2). These studies provide the first evidence for indicating that the aqueous extract prepared from the joint part of lotus root may contain novel active substances inhibiting α-amylase and α-glucosidase, proposing the possibility that the joint part extract may be able to prevent the postprandial hyperglycemia, thereby reducing effectively the risks of cardiovascular diseases as a consequence of suppressing the postprandial elevation of blood glucose levels in diabetic patients. Then, the inhibitory effects of the joint part extract on these two enzymes were chosen for the focus of our further investigation.

In preliminary study, we have determined the amounts of polyphenolic compounds contained in the aqueous extracts of lotus root, and shown that the contents of polyphenols in the joint part extract were approximately 3-times higher than those contained in the edible part extract. On the other hand, several natural materials have recently been reported to inhibit both α-amylase and α-glucosidase, and suggested that polyphenolic compounds cont-

 

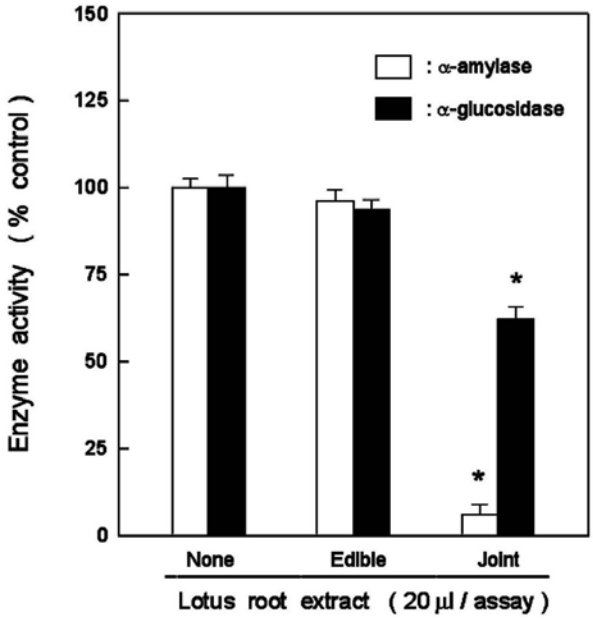


Figure 1. Effects of edible and joint part extracts on α-amylase and α-glucosidase activities. The extracts (20 μl) were added to the assay mixtures, and the enzyme activities were determined as described in the text. Results were expressed as the percent of control. Values are the mean ± SEM (*$p < 0.01$ *vs*. no extract, n = 6).

ained in these natural materials may be responsible for their inhibitory effects on these enzymes (Hogan, et al., 2010; Kim, et al., 2000; Tadera, et al., 2006). Therefore, it seems possible to consider that the different inhibitory effect on these enzymes observed between the edible and joint part extracts may be attributed to the difference in the contents of polyph-

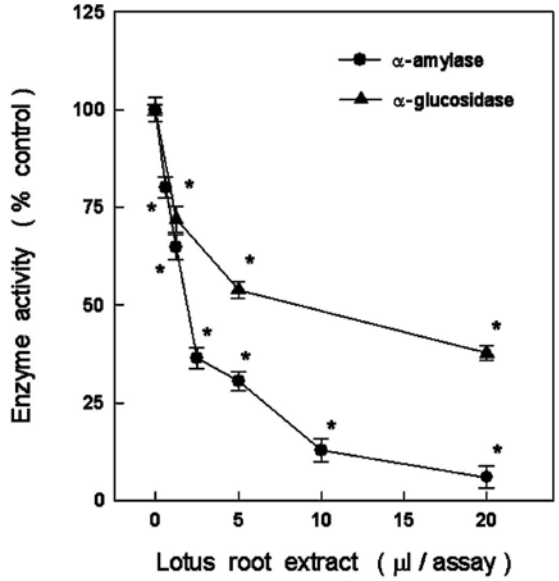


Figure 2. Concentration-dependent effects of joint part extract on α-amylase and α-glucossidase activities. Different amounts of the extract were added to the α-amylase and α-glucosidase assay mixtures, and the enzyme activities were then determined as described in the text. Results were expressed as the percent of control. Values are the mean ± SEM (*$p < 0.01$ *vs*. no extract, n = 6).

 

enolic compounds in these extracts. On the other hand, the inhibitory potencies of the joint part extract on each of these two enzymes were also shown to be markedly different, and the inhibitory effect of the extract on α-amylase was pronounced, and observed more than 2-times stronger than that on α-glucosidase (Figures 1 and 2). Therefore, it seems possible to consider that the joint part extract may contain two different active substances, each of them can specifically inhibit the respective enzymes. Alternatively, this finding can be considered to indicate that the difference in the inhibitory effect of the joint part extract between α-amylase and α-glucosidase may reflect the different properties of these enzymes themselves. However, it still remains to be elucidated, and seems absolutely necessary to be further investigated.

The joint part extract was clearly shown to reveal different inhibitory potency to α-amylase and α-glucosidase under the conditions employed to determine the enzyme activities. However, α-amylase and α-glucosidase used in these experiments were derived from porcine pancreas and yeast, respectively. Therefore, it seems possible that the different inhibitory potency of the joint part extract observed between α-amylase and α-glucosidase activities may reflect the difference in the sensitivities of these enzymes to potential inhibitory substances contained in the extract. On the other hand, it also seems conceivable that more than one active substance contained in the joint part extract may inhibit these enzymes, respectively. Therefore, it seems still necessary to further characterize the inhibitory effect of the joint part extract on these enzymes. Then, the inhibitory effect of the joint pa-

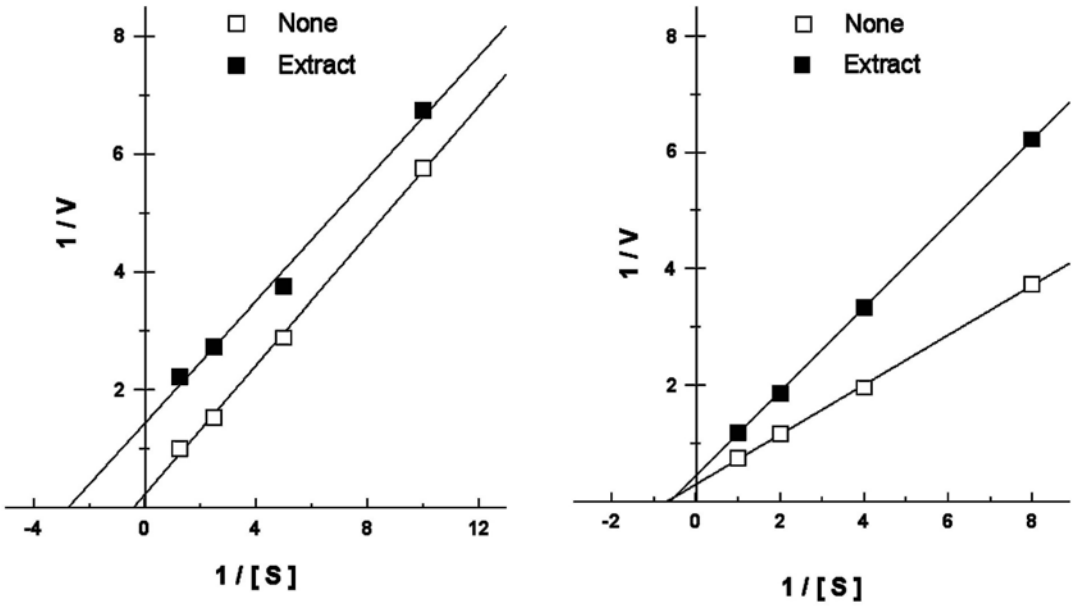


Figure 3. (Left). Lineweaver-Burk plot of inhibitory effect of joint part extract on α-amylase activity. The extract (20 μl) was added to the α-amylase assay mixture containing various concentrations of the substrate, and the enzyme activity was determined as described in the text. The enzyme kinetics was graphically represented as the double-reciprocal plot. Figure 4 (Right). Lineweaver-Burk analysis of inhibitory effect of joint part extract on α-glucosidase activity. The extract (20 μl) was added to the α-glucosidase assay mixture containing different concentrations of the substrate, and the enzyme activity was determined as described in the text. The enzyme kinetics was graphically represented as the double-reciprocal plot.

 

rt extract on α-amylase was kinetically characterized. The Lineweaver-Burk analysis indicated that the joint part extract reduced both Km and Vmax values, thus suggesting that the extract might cause the uncompetitive inhibition of this enzyme (Figure 3). On the other hand, the extract could also inhibit α-glucosidase in a concentration-dependent manner, and the Lineweaver-Burk analysis indicated that the extract caused the reduction of Vmax value without any change in Km, suggesting that the extract might inhibit this enzyme in the non-competitive manner (Figure 4). The kinetic studies on the inhibitory effects of the joint part extract indicated that the inhibition of α-amylase was uncompetitive, thus suggesting that the inhibitory substance in the extract may be able to bind only to the E-S complex formed between the enzyme and the substrate. In contrast, the extract could cause the inhibition of α-glucosidase in a noncompetitive manner, thus suggesting that the inhibitory substance can possibly bind with equal affinity to the free enzyme and the E-S complex. Therefore, it seems reasonable to consider that the joint part extract may contain several inhibitory substances, and suggest that some of which may be able to selectively act on α-amylase and others may be able to specifically interact with α-glucosidase, thereby resulting in the inhibitions of these two enzymes to the different extent of their inhibitory potencies under the assay conditions used here.

Further studies on the influence of the heat treatment on the inhibitory effect of the joint part extract were carried out to characterize the properties of putative inhibitory substances contained in the extract. As shown in Figure 5, the incubation of the joint part extract at -95°C for different periods (up to 60 min) significantly reduced the inhibitory effects on both

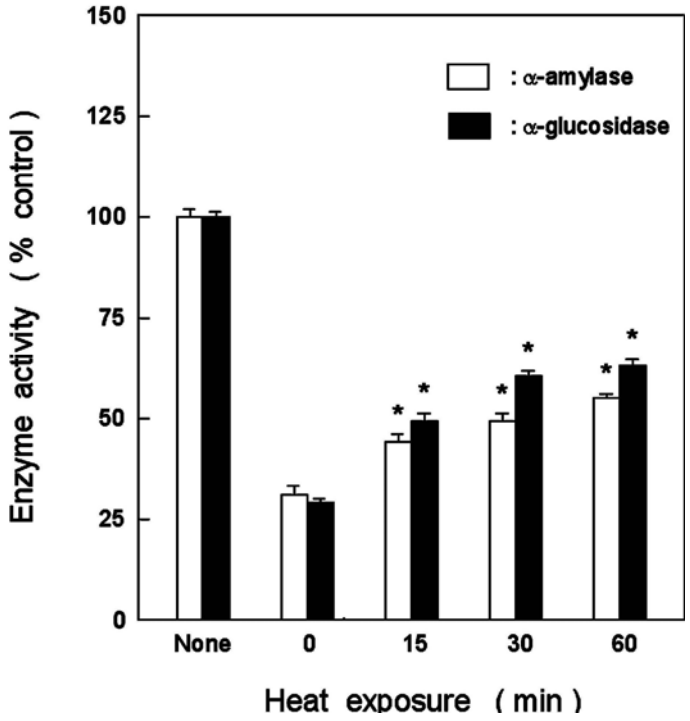


Figure 5. Inhibitory effect of heat-treated joint part extract on α-amylase and α–glucosidase activities. The extract was heated at 95 °C for different periods, and the inhibitory effects of heat treated extract on the enzyme activities were then determined as described in the text. Results were expressed as the percent of control. Values are the mean ± SEM (*$p < 0.01$ vs. non-treated, n = 6).

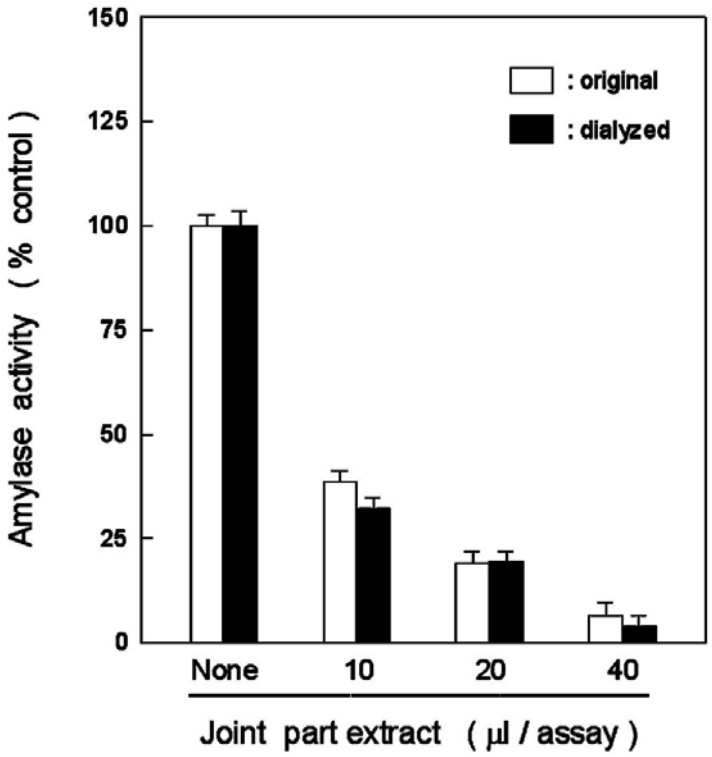


Figure 6. Inhibitory effect of dialyzed joint part extract on α-amylase activity. The extract was dialyzed against distilled water (50-volume) at 4°C for overnight (exchanged 3-times), and the inhibitory effect of the dialyzed extract on the enzyme activity was examined as described in the text. Results were expressed as the percent of control. Values are the mean ± SEM (n = 6).

α-amylase and α-glucosidase in a manner dependent on the time of heat exposure, and the potencies of the joint part extract inhibiting these respective enzymes were almost identically reduced according to the time of heat exposure, thus indicating that there was no difference observed between the thermal deactivation of the inhibitory effects of the joint part extract on these two enzymes in the *in vitro* assay systems. Accordingly, the joint part extract is considered to contain a potential thermolabile substance which can inhibit α-amylase and α-glucosidase, but the property of this inhibitory substance still remains entirely unidentified. Then, the joint part extract was subjected to the dialysis, and the inhibitory effects of the dialyzed extract on α-amylase and α-glucosidase activities were examined to estimate the molecular size of novel inhibitory substance contained in the extract. Consequently, the inhibitory effect of the joint part extract on α-glucosidase, but not α-amylase, was markedly reduced by dialyzing the extract against distilled water (Figures 6 and 7). Therefore, it seems conceivable that α-glucosidase inhibitory substance contained in the joint part extract may be small molecule, thereby being mostly removed by the dialysis. On the contrary, α-amylase inhibitory substance is speculated to be relatively large and non-dialyzable, thereby remaining in the extract even after the dialysis.

Recently, the active substances inhibiting either α-amylase or α-glucosidase have been found in a variety of natural materials, such as fruits, nuts, vegetables and other plants, thereby suggesting the potential activities of these materials to prevent the postprandial hyperglycemia. Particularly, these materials have been reported to contain considerable amo-

 

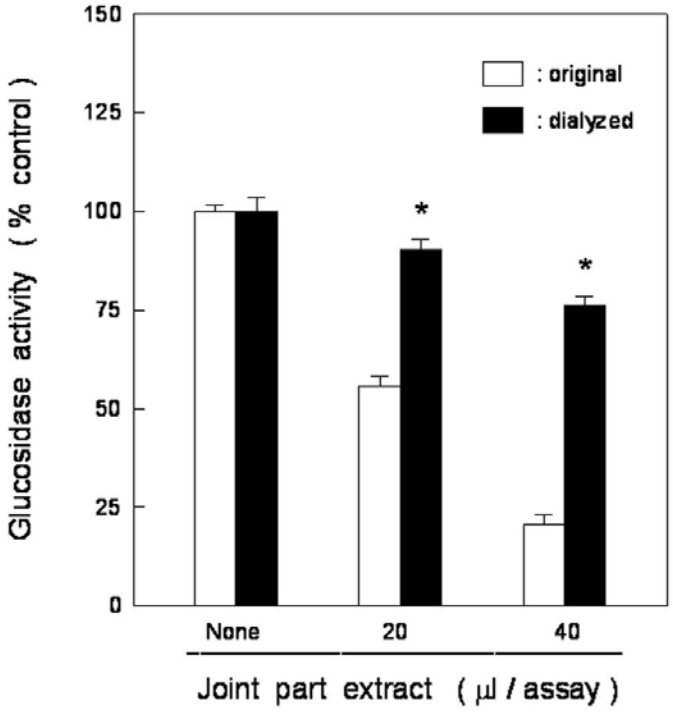


Figure 7. Inhibitory effect of dialyzed joint part extract on α-glucosidase activity. The extract was dialyzed against distilled water (50-volume) at 4°C for overnight (exchanged 3-times), and the inhibitory effect of the dialyzed extract on the enzyme activity was examined as described in the text. Results were expressed as the percent of control. Values are the mean ± SEM (*$p < 0.01$ vs. original extract, n = 6).

unts of polyphenolic compounds, and these compounds have also been suggested to be responsible for the inhibitory effects of these natural materials on α-amylase and/or α-glucosidase as well as their radical scavenging and antioxidant activities (Johnson, et al., 2011; McDougall, et al., 2005; McDougall and Stewart, 2005; Rubilar, et al., 2011). On the other hand, preliminary studies have shown that the joint part extract contains considerable amounts of polyphenolic compounds, and furthermore shown that, similarly to the extracts of other natural materials reported previously, the extract prepared from the joint part of lotus root reveals its radical scavenging and antioxidant activities in the *in vitro* experimental systems (unpublished data). Based on these findings, it seems quite possible to consider that polyphenolic compounds contained in the joint part extract may be one of the potentially active substances responsible for the inhibitory effects on α-amylase and/or α-glucosidase activities observed here.

In the *in vitro* study, the aqueous extract prepared from the joint part of lotus root was shown to inhibit both α-amylase and α-glucosidase, and suggested to contain more than one active substance, each of which was considered to separately inhibit these two enzymes. The present study are therefore considered to provide evidence for suggesting that the joint part extract may contain at least two active substances, which may be different in the molecular sizes as well as the mechanisms of their inhibitory effects on the respective enzyme, thus proposing the possibility that the joint part of lotus root may be one of the natural resources providing the potentially active substances interfering with the digestion and absorption of

 

carbohydrates, thereby preventing the postprandial elevation of blood glucose levels in patients with type 2 diabetes. However, the potentially active substance in the joint part extract is still unidentified, and the chemical properties of the lotus root extracts are therefore necessary to be further investigated.

## Conflict of Interest statement

There is no conflict of interest associated with the authors of this paper, and the fund sponsors did not cause any inappropriate influence on this work.

## References

Al-Zuhair S, Dowaidar A, Kamal H. (2010). Inhibitory effect of dates-extract on α-amylase and α-glucosidase enzymes relevant to non-insulin dependent diabetes mellitus. *Journal of Biochemical Technology* 2, 158-160.

Ceriello A. (2005). Postprandial hyperglycemia and diabetes complications: is it time to treat? *Diabetes* 54, 1-7.

Chiasson JL, Gomis R, Hanefeld M, Josse RG, Karasik A, Laakso M. (1998). The STOP-NIDDM Trial: an international study on the efficacy of an alpha-glucosidase inhibitor to prevent type 2 diabetes in a population with impaired glucose tolerance: rationale, design, and preliminary screening data. Study to Prevent Non-Insulin-Dependent Diabetes Mellitus. *Diabetes Care* 21, 1720-1725.

Hogan S, Zhang L, Li J, Sun S, Canning C, Zhou K. (2010). Antioxidant rich grape pomace extract suppresses postprandial hyperglycemia in diabetic mice by specifically inhibiting alpha-glucosidase. *Nutrition & Metabolism* 7, 71-79.

Jo SH, Ha KS, Moon KS, Lee OH, Jang HD, Kwon YI. (2011). *In vitro* and *in vivo* anti-hyperglycemic effects of omija (Schizandra chinensis) fruit. *International Journal of Molecular Sciences* 12, 1359-1370.

Johnson MH, Lucius A, Meyer T, de Mejia EG. (2011). Cultivar evaluation and effect of fermentation on antioxidant capacity and in vitro inhibition of α-amylase and α-glucosidase by highbush blueberry (Vaccinium corombosum). *Journal of Agricultural and Food Chemistry* 59, 8923-8930.

Jung HA, Kim JE, Chung HY, Choi JS. (2003). Antioxidant principles of Nelumbo nucifera stamens. *Archives of Pharmacal Research* 26, 279-285.

Kim HK, Park HR, Lee JS, Chung TS, Chung HY, Chung J. (2007). Down-regulation of iNOS and TNF-alpha expression by kaempferol via NF-kappaB inactivation in aged rat gingival tissues. *Biogerontology* 8, 399-408.

Kim JS, Kwon CS, Son KH. (2000). Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Bioscience, Biotechnology, and Biochemistry* 64, 2458-2461.

Kuo YC, Lin YL, Liu CP, Tsai WJ. (2005). Herpes simplex virus type 1 propagation in HeLa cells interrupted by Nelumbo nucifera. *Journal of Biomedical Science* 12, 1021-1034.

la Cour B, Molgaard P, Yi Z. (1995). Traditional Chinese medicine in treatment of hyperlipidaemia. *Journal of Ethnopharmacology* 46, 125-129.

Ling ZQ, Xie BJ, Yang EL. (2005). Isolation, characterization, and determination of antioxidative activity of oligomeric procyanidins from the seedpod of Nelumbo nucifera Gaertn. *Journal of Agricultural and Food Chemistry* 53, 2441-2445.

Liu CP, Kuo YC, Shen CC, Wu MH, Liao JF, Lin YL, Chen CF, Tsai WJ. (2007). (S)-armepavine inhibits human peripheral blood mononuclear cell activation by regulating Itk and PLCgamma activation in a PI-3K-dependent mnner. *Journal of Leukocyte Biology* 81, 1276-1286.

 

McDougall GJ, Shpiro F, Dobson P, Smith P, Blake A, Stewart D. (2005). Different polyphenolic components of soft fruits inhibit alpha-amylase and alpha-glucosidase. *Journal of Agricultural and Food Chemistry* 53, 2760-2766.

McDougall GJ, Stewart D. (2005). The inhibitory effects of berry polyphenols on digestive enzymes. *BioFactors* 23, 189-195.

Mukherjee D, Khatua TN, Venkatesh P, Saha BP, Mukherjee PK. (2010). Immunomodulatory potential of rhizome and seed extracts of Nelumbo nucifera Gaertn. *Journal of Ethnopharmacology* 128, 490-494.

Mukherjee PK, Saha K, Pal M, Saha BP. (1997). Effect of Nelumbo nucifera rhizome extract on blood sugar level in rats. *Journal of Ethnopharmacology* 58, 207-213.

Rubilar M, Jara C, Poo Y, Acevedo F, Gutierrez C, Sineiro J, Shene C. (2011). Extracts of Maqui ( Aristotelia chilensis ) and Murta ( Ugni molinae Turcz.): sources of antioxidant compounds and α-Glucosidase/α-Amylase inhibitors. *Journal of Agricultural and Food Chemistry* 59, 1630-1637.

Sinha S, Mukherjee PK, Mukherjee K, Pal M, Mandal SC, Saha BP. (2000). Evaluation of antipyretic potential of Nelumbo nucifera stalk extract. *Phytother Res* 14, 272-274.

Subramanian R, Asmawi MZ, Sadikun A. (2008). *In vitro* α-glucosidase and α-amylase Enzyme inhibitory effects of *Andrographis paniculata* extract and andrographolide. *Acta Biochimica Polonica* 55, 391-398.

Tadera K, Minami Y, Takamatsu K, Matsuoka T. (2006). Inhibition of α-glucosidase and α-amylase by flavonoids. *Journal of Nutritional Science and Vitaminology* 52, 149-153.

Talukder MJ, Nessa J. (1998). Effect of Nelumbo nucifera rhizome extract on the gastrointestinal tract of rat. *Bangladesh Medical Research Council Bulletin* 24, 6-9.

Tsujita T, Takaku T. (2008). Mechanism of the inhibitory action of chestnut astringent skin extract on carbohydrate absorption. *Journal of Nutritional Science and Vitaminology* 54, 416-421.

Tsujita T, Takaku T, Suzuki T. (2008). Chestnut astringent skin extract, an α-amylase inhibitor, retards carbohydrate absorption in rats and humans. *Journal of Nutritional Science and Vitaminology* 54, 82-88.

Tundis R, Loizzo MR, Menichini F. (2010). Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. *Mini-Reviews in Medicinal Chemistry* 10, 315-331.

Xiao Z, Storms R, Tsang A. (2006). A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. *Analytical Biochemistry* 351, 146-148.

Yang WM, Shim KJ, Choi MJ, Park SY, Choi BJ, Chang MS, Park SK. (2008). Novel effects of *Nelumbo nucifera* rhizome extract on memory and neurogenesis in the dentate gyrus of the rat hippocampus. *Neuroscience Letters* 443, 104-107.

 