Suppressive Effect of a Hot Water Extract of Adzuki Beans (Vigna angularis) on Hyperglycemia after Sucrose Loading in Mice and Diabetic Rats

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Received April 19, 2004; Accepted July 1, 2004

A hot water extract obtained by boiling adzuki beans (Vigna angularis) to produce bean paste for Japanese cake showed inhibitory activity against alpha-glucosidase, alpha-amylase, maltase, sucrase, and isomaltase after HP-20 column chromatography. The IC50 values for each hydrolylase were 0.78 mg/ml (alpha-glucosidase), 2.45 mg/ml (maltase), 5.37 mg/ml (sucrase), and 1.75 mg/ml (isomaltase). The active fraction showed potential hypoglycemic activity in both normal mice and streptozotocin (STZ)-induced diabetic rats after an oral administration of sucrose, but did not show any effect on the blood glucose concentration after glucose administration, suggesting that the active fraction suppressed the postprandial blood glucose level by inhibiting alpha-glucosidase and alpha-amylase, irrespective of the endogenous blood insulin level.

Key words: adzuki bean (Vigna angularis); alpha-glucosidase; alpha-amylase; potential hypoglycemic activity

Adzuki (Vigna angularis) is a very important bean in the Far East. It is used as a diuretic, antidote, and remedy for dropsy and beriberi in traditional Chinese medicine, and is also used for the production of traditional confectionery (wagashi), e.g., youkan manju and ananatoo, in Japan.

When adzuki beans are used for confectionery, they are boiled in a cooker to afford a hot water extract as a by-product which is known to contain active ingredients, but is generally discarded. The hot water extract is known to contain polyphenols like tannins, phytic acid, and saponin, but its effective use has not been well examined.

Material and Methods

Preparation of the test materials. The hot water extract was obtained by boiling the beans of adzuki which had been harvested in Tokachi (Hokkaido, Japan). The extract was concentrated in vacuo, and ca. 15 liter from ca. 2.5 kg of adzuki beans gave ca. 30 g of a concentrate which was subjected to open column chromatography on Diaion HP-20 (Mitsubishi Chemical Co., Tokyo, Japan; column size 5φ x 300 mm). The loaded column was stepwise eluted with distilled water and 40%, 60%, and 80% ethyl alcohol. The respective fractions were evaporated to dryness.

Alpha-glucosidase and alpha-amylase inhibitory assay. An enzyme solution was prepared from rat small intestinal acetone powder (Sigma, St. Louis, Missouri, U.S.A.). Crude enzyme solutions were prepared by following the manufacturer’s instructions. Briefly, 1 g of the rat small intestinal acetone powder was mixed with 9 ml of a 56 mM maleate buffer (pH 6.0), homogenized on ice, and centrifuged at 3,000 rpm (4°C, 10 min). After centrifugation, the supernatant was used as a crude enzyme solution. This crude enzyme solution was 20-times diluted with a 56 mM maleate buffer for the maltase reaction, 2-times diluted for the sucrase and isomaltase reactions, and 4-times diluted for the glucoamylase reaction. Alpha-amylase (from human saliva and porcine pancreas, Sigma) was used to prepare an enzyme solution at 267.5 unit/ml with a 0.1 M sodium phosphate buffer (pH 7.0).

The inhibitory effect of each fraction on the alpha-glucosidase activity was next examined. Each sugar substrate was dissolved separately in the 56 mM maleate buffer to 2% w/v. An enzyme solution (50 μl) and a fraction solution (50 μl) were pre-incubated together for 5 min (37°C, pH 6.0), and then the enzyme reaction was started by adding each sugar substrate solution (2% w/v, 100 μl). Each fraction solution diluted with the 56 mM...
maleate buffer was added in the concentration range of 0.01–100 mg/ml. As a positive control, the 56 mM maleate buffer was added in place of the fraction, and as a blank, a degenerated enzyme solution was added in place of the crude enzyme solution. The enzymatic reaction was terminated by boiling the reaction tube for 10 min. The reaction mixture was centrifuged at 3,000 rpm for 10 min at room temperature. The glucose produced in the reaction was measured with a commercial assay kit (Glucose C II-test, Wako Pure Chemicals, Osaka, Japan).

The rate of carbohydrate decomposition was calculated as a percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The rate of prevention was calculated by the following formula:

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\text{Inhibition rate} \% = \left( \frac{\text{(Amount of glucose produced by the positive control)} - \text{(Amount of glucose produced after the addition of each fraction)}}{\text{(Glucose production value of blank)}} \right) \times 100.
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The inhibitory effect of the adzuki extract on \(\alpha\)-amylase was also examined, in accordance with the method already described, but with some modifications. Soluble starch was dissolved in the 56 mM maleate buffer (pH 6.0) to 4\% w/v. The enzyme solution (20 \(\mu\)l, 5.35 unit) and each fraction solution (100 \(\mu\)l) were pre-incubated together for 5 min at 37\°C and pH 6.0, and then the enzyme reactions were started by adding 4\% soluble-starch solutions (100 \(\mu\)l). The maltose produced was measured by high-performance liquid chromatography, and the enzyme activity was measured. The rate of enzyme inhibition was calculated by the same formula as that for \(\alpha\)-glucosidase, as already described.

The conditions set for HPLC were as follows: pump, Jasco PU-1580 (Nihon Bunko, Tokyo, Japan); column, Shodex Asahipak NH2-P-50-4 (Showadenko, Tokyo, Japan); column temp., 35\°C; mobile phase, 75\% CH3CN; flow rate, 0.8 ml/min; detection, RI (Nihon Bunko, Tokyo, Japan); sample injection volume, 20 \(\mu\)l.

**Oral glucose tolerance test on normal mice.** Six-week-old male mice (ICR, Japan SLC, Hamamatsu, Japan) were kept under a light schedule of 0800–2000 h at 22 ± 3\°C. The mice had been allowed access to water and a non-purified laboratory diet (MF, Oriental Yeast Co.) for one week to accustom them to their surroundings. After overnight fasting (18 h) at seven weeks of age, the rats were orally gavaged with a mixture of sucrose or glucose (2 g/kg body weight) and the 40\% ethanol fraction (100 mg, 500 mg/kg body weight). Blood samples (200 \(\mu\)l) were taken from the lateral tail vein at various times during 0–120 min. The blood glucose concentration was measured by the glucose oxidase method with a glucose analyzer, and the serum insulin concentration was determined with an insulin measurement kit. Physiological saline and tolbutamide, a known sulfonylurea hypoglycemic agent (100 mg/kg body weight), were administered instead of the 40\% ethanol fraction, and the same steps were performed. These steps, with a similar solution of physiological saline and sucrose or glucose, were performed on normal rats without the 40\% ethanol fraction.

These studies were approved by the Mie University Animal Use Committee, and the animals were maintained according to the guidelines of Mie University for the care of laboratory animals.

**Oral glucose tolerance test on rats with streptozotocin (STZ)-induced diabetes.** Five-week-old male Wistar-strain rats (Japan SLC, Hamamatsu, Japan) were used in palace of six-week-old normal mice. They were kept under an automatic light schedule of 0800–2000 h at 22 ± 3\°C. The rats had free access to water and a non-purified laboratory diet (MF, Oriental Yeast Co.) for one week to accustom them to their surroundings. At six weeks of age, diabetes was induced by administering STZ (45 mg/kg body weight) in a 50 mM citrate buffer (pH 4.5) via intraperitoneal injection. Seven days after the STZ administration, blood samples were taken without fasting, and the blood glucose level was measured. Individual mice with a level above 200 mg/dl of blood glucose were used for the experiment on oral glucose tolerance. After overnight fasting (18 h) at seven weeks of age, the rats were orally gavaged with a mixture of sucrose or glucose (2 g/kg body weight) and the 40\% ethanol fraction (100 mg, 500 mg/kg body weight). Blood samples (200 \(\mu\)l) were taken from the lateral tail vein at various times during 0–120 min. The blood glucose concentration was measured by the glucose oxidase method with a glucose analyzer, and the serum insulin concentration was determined with an insulin measurement kit. Physiological saline and tolbutamide, a known sulfonylurea hypoglycemic agent (100 mg/kg body weight), were administered instead of the 40\% ethanol fraction, and the same steps were performed. These steps, with a similar solution of physiological saline and sucrose or glucose, were performed on normal rats without the 40\% ethanol fraction.

**Statistical analysis.** The oral sucrose or glucose tolerance level of normal mice and rats with streptozotocin (STZ)-induced diabetes is expressed as the mean ± S.E. for \(n = 6\). Differences among the three groups at various times were analyzed by Duncan’s multiple-range test; points without common letters in Figs. 3 and 4 differ significantly (\(p < 0.05\)). The oral maltose or soluble starch tolerance level of normal mice is expressed as the mean ± S.E., and a subsequent inspection of the statistical significance of the difference of means was evaluated by Student’s T test between the two groups at the level of \(p < 0.05\).
Results

Alpha-glucosidase and α-amylase inhibitory activities
When the inhibitory activities on α-glucosidase and α-amylase were investigated, the 40% ethanol fraction (EtEx.40) showed the highest activity (data not shown). The amount of glucose produced by the maltase, sucrase, isomaltase, or glucoamylase reaction and the amount of maltose produced by α-amylase (from human saliva) or α-amylase (from porcine pancreas) respectively decreased to 36, 55, 37.5, 5.5, 12, and 57% when incubated for 120 min with EtEx.40. The IC_{50} value for EtEx.40 was measured for each enzyme reaction as follows: 2.45, 5.37, 1.75, 12.24, 0.78, and 7.8 mg/ml (Fig. 1).

Oral glucose tolerance test on normal mice
The effect of EtEx.40 on the postprandial blood glucose level and serum insulin level was examined with sucrose- and glucose-loaded mice. When EtEx.40 was orally administered simultaneously with sucrose to the mice, the postprandial blood glucose level was lower 15 and 30 min after the administration (p < 0.05). This suppressive effect was dose-dependent (Fig. 2a). Moreover, insulin secretion also tended to significantly decrease with the administration of EtEx.40 at 500 mg/kg mouse body weight (Fig. 2b). There was no significant difference in the levels of postprandial blood glucose and serum insulin (p < 0.05, Fig. 3a, b) when sucrose was replaced by glucose. The postprandial blood glucose level was also significantly decreased when the mice were orally administered with maltose or soluble starch together with EtEx.40 at 500 mg/kg body weight. The lower level at 15 and 30 min after the administration was particularly notable (Fig. 4).

Oral glucose tolerance test on rats with STZ-induced diabetes
The effect of EtEx.40 on the postprandial blood glucose and serum insulin level was also examined for

Fig. 1. Dose–Response Curves for the Inhibitory Effect of EtEx.40 on the Activities of Maltase, Sucrase, Isomaltase, Glucoamylase, and α-Amylase (from human saliva (H.S.) and from porcine pancreas (P.P.)).

Fig. 2. Effect of EtEx.40 on the Postprandial Blood Glucose (a) and Serum Insulin (b) Levels in Sucrose-Loaded Mice.
EtEx.40 and 2 g of sucrose/kg were simultaneously given by oral administration to 7-week-old ICR mice after overnight (15 h) fasting. Blood samples were taken at 0, 15, 30, 60, 90, and 120 min after the loading. Each point represents the mean ± S.E. (n = 6). ●, Control; △, 100 mg/kg; □, 500 mg of EtEx.40/kg. Values not sharing a common letter are significantly different among the groups at the corresponding times (p < 0.05, Duncan’s multiple-range test).
the rats with STZ-induced diabetes. The glucose levels before and after administering sucrose at 2 g/kg of rat body weight and EtEx.40 at 100 and 500 mg/kg body weight were 240 ± 12.97, 259 ± 23.91, and 293 ± 11.89 mg/dl, respectively. No significant difference was found when 100 mg/kg body weight of EtEx.40 was administered. However, the 500 mg/kg body weight EtEx.40 group tended to show a suppressed postprandial blood glucose level compared to the group given physiological saline at 30 and 60 min after the administration. On the other hand, the blood glucose level after the administration of 100 and 500 mg/kg body weight to the EtEx.40 group was 292 ± 21.76 and 263 ± 32.84 mg/dl, respectively. In the case of glucose administration, EtEx.40 did not show any significant effect.

**Discussion**

Our data show that EtEx.40 had an inhibitory effect on α-glucosidase and α-amylase that are both present in
the small intestinal mucosa, and that the administration of EtEx.40 resulted in a lower postprandial blood glucose level. Rats with STZ diabetes constitute an experimental diabetes model of a lack of insulin due to damaged beta cells in the pancreas. In this study, EtEx.40 did not stimulate insulin secretion or reinforce insulin action. Perhaps these results indicate that suppression of the postprandial blood glucose level by EtEx.40 was mainly due to disaccharidase inhibition.

Acarbose\(^1\) and voglibose\(^2\) have been developed and clinically used for diabetes therapy. On the other hand, \(\alpha\)-xylose has attracted attention as a food additive with sucrase inhibitory activity.\(^{17}\) Research has recently been conducted on \(\alpha\)-glucosidase inhibitors obtained from vegetables, on the assumption that these could suppress the postprandial blood glucose level.\(^{18-20}\) A guava leaf extract has been reported to be an \(\alpha\)-glucosidase inhibitor. The IC\(_{50}\) values for the guava leaf extract against maltase and sucrase were 2.6 and 3.1 mg/ml, respectively. The inhibitory activity of EtEx.40 against disaccharidase was equivalent to the effect of the guava leaf extract. Although acarbose and voglibose were both effective in depressing the concentration of glucose, the Ministry of Health, Labor, and Welfare of Japan has reported that these medicines induced abnormalities in the hepatic enzymes. However, \(\alpha\)-glucosidase inhibitors from plants, including EtEx.40, are free of such side effects. Deguchi et al. have reported that the postprandial blood glucose level was significantly decreased by an oral administration of a guava leaf extract to mice 30 min before sucrose loading.\(^{21}\) In this present study, the postprandial blood glucose level of normal mice and rats with STZ-induced diabetes was also suppressed by an oral administration of EtEx.40. It appears that this suppression of the postprandial blood glucose level was due to intestinal \(\alpha\)-glucosidase inhibition.

Kaneko has reported that the blood glucose level was not directly influenced by a rapid change in blood insulin level.\(^{22}\) Thus, it seems that significant suppression of insulin secretion had no effect on the blood glucose level when the sugars and EtEx.40 were simultaneously given. The postprandial blood glucose level 60 min after administration was higher in the EtEx.40 group than in the physiological saline group. This appears to have been due to the delay in sugar digestion caused by EtEx.40. It is possible that EtEx.40 markedly suppressed the initial amount of insulin secretion by suppressing the rise in blood glucose level after loading with sugar. Excessive insulin secretion after feeding is stimulated by the accumulation of visceral fat, and causes obesity. Obesity increases the insulin resistance and causes diabetes as a result. Perhaps EtEx.40 is effective in preventing diabetes in that it controls insulin secretion while also controlling the blood glucose level.

It has recently been reported that tea polyphenols inhibited glucose transporter of small intestinal epithelial cells.\(^{23,24}\) EtEx.40 might show glucose transporter-inhibition activity, because EtEx.40 includes about 50% polyphenols. In addition, Thompson et al. have indicated the possibility that polyphenols controlled the rise in blood glucose level when humans were fed with a fixed amount of carbohydrates with food, including legumes, because a negative correlation was indicated by the polyphenol content and glycemic index.\(^3\)

This result suggests that EtEx.40 decreased the postprandial blood glucose level by disaccharidase inhibition. It appears that this effect is associated with polyphenols in EtEx.40. These components were involved and then influenced the postprandial glucose level. There is a pressing need to identify the disaccharidase-inhibiting substance that brings about suppression of the postprandial blood glucose level by EtEx.40. In order to see the effect of EtEx.40 on most insulin non-dependence diabetes (NIDDM), which involves 90% of the number of diabetics, we need to investigate this by using KK-A\(^y\) mice which is a natural development of the symptoms diabetes model.

References

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