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An ethanol extract (EPE) was prepared from defatted seeds of Evening-primrose (*Oenothera biennis* L.). In this study, we examined the effect of EPE on postprandial blood glucose level and its active components. Detected polyphenols components of EPE were penta-*O*-galloyl-β-D-glucose (PGG) 2.7%, gallic acid 3.1%, (+)-catechin 3.4%, procyanidin B1 and procyanidin B3 1.5%. 41.4% of total proanthocyanidin (PAC) was contained in EPE. Then, we examined the effect of these components of EPE on inhibitory action against α-glucosidase *in vitro* and on the postprandial blood glucose level using rats. The results suggested that 1) the suppression of postprandial hyperglycemia was caused by inhibition of α-glucosidase activity due to EPE, 2) PAC was the main active component of EPE. (Received Nov. 21, 2002; Accepted Jan. 21, 2003)

It is estimated that there are 13.7 million diabetic patients in Japan, that is about one in ten of the entire population[1], including those who are fear for diabetic. Over 90 percent of the diabetic patients are classified as type 2 diabetes which starts with a temporal high postprandial blood glucose level. It is suggested that patients experience consistent high postprandial blood glucose level, and eventually that their fasting blood glucose levels become high as diabetes worsens[2]. Therefore, it is important to normalize the rise of postprandial blood glucose level for primary prevention of diabetes. Clinically, an agent that controls the decomposition of disaccharide into monosaccharide (α-glucosidase inhibitor) has been known to be effective to prevent the sudden rise of postprandial blood glucose level[3].

Evening primrose (*Oenothera biennis* L.) is a plant that belongs to *oenothera, onagraceae* and it is North America origin. Pickled or boiled root have been used as an appetite stimulant[4]. Recently, it was discovered that evening primrose seed oil is rich in γ-linolenic acid, and therefore, it is expected that the oil moderates combat obesity, diabetes, and premenstrual syndrome (PMS)[5]. Although little attention was paid to components of evening primrose other than oil, some recent reports indicated the antioxidant activity of polyphenols contained in evening primrose seeds[6)-10)].

Today, the discovery of plant-derived polyphenol's inhibitory effect against glucose digestive enzymes, such as α-glucosidase, attracts people's attention[11)12)]. A number of studies have been conducted in this field because ingestion of food with this action with meals is considered to be promising to prevent diabetes. One of the researches suggested that guava leaf polyphenols prevent postprandial blood glucose level rise by inhibiting α-glucosidase[11)]. We have confirmed high concentration of polyphenols in the extract of evening primrose seeds (evening primrose extract), obtained by concentrating and drying aqucious ethanol-extract of defatted evening primrose seeds. This implies that evening primrose extract has a potential activity to control the rise of postprandial blood glucose level with polyphenols, by inhibiting α-glucosidase.

We conducted an *in vitro* test to clarify evening primrose extract's activity to control the rise of blood glucose level and its active components, analyze composition of polyphenols contained in the extract, and examine if the extract and contained polyphenols have the inhibitory action against α-glucosidase. Moreover, we conducted a sugar tolerance test on rats concerning evening primrose extract and its components with α-glucosidase inhibition activity in order to confirm if they control the rise of blood glucose level after sugar is loaded.

Test Methods

1. Test material

We used a material ethanol-extracted from evening primrose (*Oenothera biennis* L.) seeds as evening primrose extract (product name: Evening Primrose Extract-P, manufactured by Oryza Oil & Fat Chemical). We added 500 ml of 70 percent ethanol to 100 g of defatted and crushed evening primrose seeds, agitated it for four hours at 51ºC, filtered it, concentrated and dried the filtered solution to obtain approximately 6 g of evening primrose extract. 100 g of evening primrose extract contains 1.5 g water, 6.6 g protein, 4.9 g fat, 3.7 g ash, and 1.0 g fiber other than polyphenols. (Analysis was contracted to the Japan Food Research Laboratories. Report number: 300080303-002).

2. Analysis of polyphenols components contained in evening primrose extract

(1) Quantitative determination of the total polyphenol content

We analyzed the total content of polyphenols contained in evening primrose extract using the Folin-Ciocalteu method[13]. We added 2.5 ml of x10-diluted Folin-Ciocalteu reagent (Kishida Chemical) to 0.5 ml of evening primrose extract solution (100 μg/ml, 10% methanol solution), added 2 ml of 7.5% sodium carbonate solution within a time between 30 seconds and 8 minutes, and agitated it well. We left it for two hours at a room temperature and measured the absorbance at 765 nm (Hitachi U-3200 spectral photometer) to calculate the total polyphenol content as the equivalent of gallic acid (SIGMA).

(2)Quantitative determination of the total proanthocyanidin (PAC) content

We analyzed the total content of PAC contained in evening primrose extract using a modified Porter method[14]. We added 6 ml of n-butanol-concentrated hydrochloric acid (95:5) and 0.2 ml of 2% $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$ solution (prepared with 2N hydrochloric acid) to 1 ml of evening primrose extract solution (100 μg/ml, methanol solution) in this order and agitated it. We heated the compound solution in a covered test tube for 40 minutes at 95ºC, cooled it, and measured the absorbance at 550 nm (Hitachi U-3200 spectral photometer) to calculate the total PAC content as the equivalent of procyanidin B2 (PB2, Asahi Breweries). We used non-heated samples as control products.

(3) Analysis of single polyphenols contained in evening primrose extract

We analyzed gallic acid, (+)- catechin, and penta-galloyl glucose (PGG) contained in evening primrose extract using high-performance liquid chromatography (HPLC) with modifying the method used by the research group lead by Mr. Yoshida[15]. Analytical conditions of HPLC were as follows.

Detector: SPD-10A detector (Shimadzu Corporation)

Data processing instrument: C-R7 Aplus (Shimadzu Corporation)

Column: Develosil ODS-UG-5 column (4.6 mm x 250 mm, Nomura Chemical)

Mobile phase: 0.05 M H_3PO_4/0.05 M Na_2HPO_4/ethanol/ethyl acetate=40/40/14/5

Speed of flow: 0.5 ml/min., Detection wavelength: 280 nm, Column tank temperature: 40ºC

Sample injected: 5 μl (Samples are prepared with 80% ethanol.)

To identify garllic acid and (+)- catechin, we used a commercially available standard product (SIGMA). To immobilize PGG, we used a synthesized material according to the method taken by the research group lead by Mr. Yoshizawa[16]. We have confirmed that the main component of the synthesized material is the same substance as PGG isolated from evening primrose extract through instrumental analysis. (Analysis was contracted to Toray Research Center; Report no. S105010-01)

We analyzed single polyphenol components other than the above using HPLC according to the method taken by the research group lead by Mr. Yanagida[17]. Analytical conditions of HPLC were as follows.

Detector: L-7420 UV-VIS detector (Hitachi)

Data processing instrument: D-7000 HPLC System Manager (Hitachi)

Column: Inertsil ODS-3 column (4.6 mm I.D. x 150 mm, GL Science)

Mobile phase: Solution A: 0.01 M KH_2PO_4 (prepared to pH 2.0 with H_3PO_4)/methanol=80/20

Solution B: 0.01 M KH_2PO_4 (prepared to pH 2.0 with H_3PO_4)/methanol=50/50

Gradient conditions: 0 → 10 min. Solution A: 100%, 10 → 50 min.

Solution A: 100% → Solution B: 100%, 50 → 65 min. Solution B: 100%

Speed of flow: 1.0 ml/min., Detection wavelength: 280 nm, Column tank temperature: 30ºC

Sample injected: 10 μl (Samples are prepared with 50% methanol.)

To identify each polyphenol component, we used commercially available standard products; (-)- epicatechin (SIGMA), ellagic acid (Wako Pure Chemical), procyanidin B1 (PB1, Asahi Breweries), PB2 (Asahi Breweries), procyanidin B3 (SIGMA), and procyanidin C1 (PC1, Asahi Breweries).

3. Preparation of a fraction with high-concentration of PAC (PAC fraction)

We prepared the PAC fraction used in the in vitro test by the following operation. We added 6 ml of distilled water to approximately 150 mg of evening primrose extract to make it suspended solution. We added 6 ml of ethyl acetate to it, agitated, and centrifuged, and removed the ethyl acetate layer. We then added 6 ml of ethyl acetate and conducted the same operation twice. We made the obtained aqueous layer absorb to Sep-Pac Plus tC18 ENV cartridge (Waters) preprocessed with 15 ml of 60% acetone and then 15 ml of distilled water. After washing with 15 ml of distilled water and eluted with 10 ml of 60% acetone and concentrated and dried it to obtain the PAC fraction (yield 19%). We measured the total polyphenol and PAC contents within the PAC fraction just like the measurement of evening primrose extract. We also analyzed components of single polyphenols in the PAC fraction same as the analysis of evening primrose extract. We prepared the PAC fraction used in the sucrose tolerance test on rats by the operation above from 60 g of evening primrose extract.

4. Measurement of inhibitory action of evening primrose extract and its polyphenol components against α-glucosidase

(1) Measurement of α-glucosidase inhibitory action

We measured altering the method taken by the research group lead by Konishi[18]. We added 0.1 M phosphoric acid buffer of pH 7.0 (Wako Pure Chemical) approximately in ten times volume to rat's

small intestine acetone powder (SIGMA) and ultrasonic-processed and centrifuged it to create enzymatic liquid from the supernatant. We diluted 4-methylumbelliferyl-α-D-glucopyranoside (SIGMA) to 0.2 mM with phosphoric acid buffer to use it as the substrate solution. As samples, we used evening primrose extract, PAC fraction, synthetic PGG, garlic acid (SIGMA), (+)- catechin (SIGMA), and PB1 (Asahi Breweries). We dissolved each sample in dimethylsulfoxide (DMSO) to make sample concentrate solutions and created diluted sample solutions of five different concentrations in two time increments to make 4% DMSO-containing phosphoric acid buffer. We created enzyme reactions using a 96-hole plate used for fluorescence assay (NUNU) with the method below. We added the substrate solution (25 μl/well) to the diluted sample solutions (50 μl/well), preheated them at 37ºC for 10 minutes and added enzyme solution (25 μl/well) to create enzyme reaction at 37ºC for 30 minutes. (Final enzyme concentration: 1 mg protein/ml, Final substrate concentration: 0.05 mM) After the reaction, we added a stop solution (0.2 M Na_2CO_3 Buffer, 100 μl/well) to stop the reaction and measured the fluorescence value (excitation wavelength 366 nm, emission wavelength 450 nm) using a micro plate spectrophotofluorometer (Molecular Device SPECTRA MAX GEMINIXS).

(2) Data processing

We set the fluorescence values when enzyme reaction described above is caused using sample solutions and the control solution (4% DMSO-containing phosphoric acid buffer) to Lx and L_0. Blank ($B_{0)}$ is the fluorescence value when the control solution is used as a sample and the timing to add the enzyme solution was delayed to after adding the stop solution. Inhibitory rates of sample solutions were determined to be $(L_0-L_x)/(L_0-B_0)$. We calculated the IC_{50} value using the statistical analysis software SAS (Statistical Analysis System). We calculated the activity contribution rate by (IC_{50} value of evening primrose extract) x (polyphenol component content (%)) / (IC_{50} value of polyphenol component).

5. Carbohydrate tolerance test on rats

(1) Test animals

We raised 5-week old male Wistar rats for one to two weeks beforehand and used them in our carbohydrate tolerance test (6 rats/group).

(2) Sucrose tolerance test

We had the rats fast for 16 hours, took their blood through veins on their tails, and orally gave them evening primrose extract suspended in distilled water (0, 500, 1,000 mg/10 ml), PAC fraction (0, 100, 500, 1,000 mg/10 ml), or synthetic PGG (0, 100, 500 mg/10 ml) through the stomach so that the content of each sample was 10 ml/kg body weight wise. Five minutes later, we orally gave them 2 g/5 ml of sucrose dissolved in distilled water as carbohydrate so that the content was 5 ml/kg body weight wise. We took the rats' blood 30, 60, 90, and 120 minutes later after sucrose was given in order to measure their blood glucose level. We took their blood through veins on their tails unanesthetized using postheparin hematocrit capillary tubes. After centrifugal separation, we obtained plasma. We stored the plasma in ice until measurement. We used Determiner GL-E (Kyowa Medics) for the measurement of blood glucose level. We conducted a test separately on each sample; evening primrose extract, PAC fraction, and synthetic PGG.

(3) Glucose tolerance test

We carried out a glucose tolerance test with the same operation as the sucrose tolerance test except that we used evening primrose extract (0, 500, 1,000 mg/ 10 ml) as a sample and 2 g/ 5 ml of glucose as carbohydrate.

(4) Statistical analysis

Blood glucose levels are indicated by the average value ± standard deviation. We compared blood glucose levels of groups that took samples with different concentrations and the levels of the group that took 0 mg/kg of samples using the Dunnett method. To verify significant difference, we employed the two-tailed test and set the significance level to 5% or lower.

Results

1. Composition of polyphenol components contained in evening primrose extract

Table 1 shows contents of polyphenol components contained in evening primrose extract. We learned that the total polyphenol content in evening primrose extract is 63.1% and total PAC content is 41.4%. Concerning single polyphenol components, we confirmed the existence of PGG 2.7%, gallic acid 3.1%, and (+)-catechin 3.4%. Fig. 1 shows HPLC chromatogram of them. Concerning other single polyphenol components, we confirmed the existence of PB1 and PB3 (1.5% in total), though, we could not confirm the existence of ellagic acid, (-)- epicatechin, PB2, or PC1.

2. Inhibitory action of evening primrose extract and its polyphenol components against α-glucosidase

We measured α-glucosidase inhibitory action of evening primrose extract and polyphenol components that were found in the extract. Table 2 shows the IC_{50} value for α-glucosidase activity of evening primrose extract and its polyphenol components. IC_{50} value of evening primrose extract is 0.34 mg/ml. Among the polyphenol

Table 1 Contents of Polyphenol Components Contained in Evening Primrose Extract

Component	Content in Evening Primrose Extract (%)
PGG	2.7
Gallic acid	3.1
(+)- catechin	3.4
(-)- epicatechin	— a)
Ellagic acid	—
PB1+PB3	1.5 b)
PB2	—
PC1	—
Total PAC	41.4
Total polyphenol	63.1

a): Smaller than the minimum detectable amount
b): Shown in a total value, because peaks of PB1 and PB3 could not be separated.

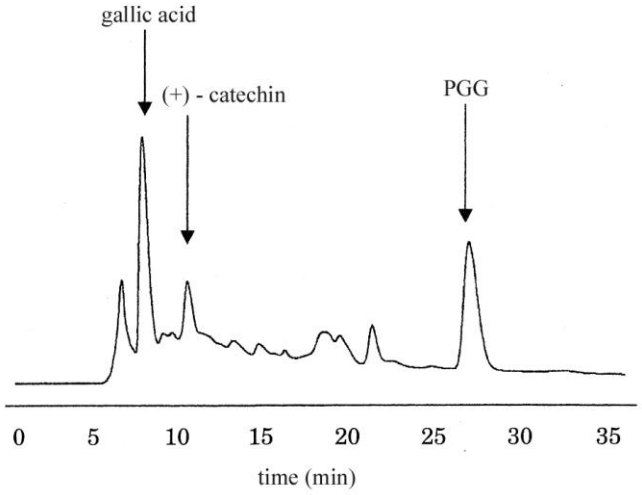

Fig. 1 HPLC Chromatogram of Evening Primrose Extract

components contained in evening primrose extract, PGG showed the highest α-glucosidase inhibitory action, 3.4 times higher than evening primrose extract. PAC fraction's α-glucosidase inhibitory action was 1.3 times higher than evening primrose extract. PAC fraction's percentages in total polyphenol content, total PAC content, and total polyphenol content of the total PAC are 85.1%, 80.8%, and 94.9% respectively. PAC fraction did not include single polyphenol components that were found in evening primrose extract in our test (PGG, gallic acid, (+)- catechin, PB1 and PB3).

We then calculated the activity contribution rate of each polyphenol component from their contents in evening primrose extract and α-glucosidase inhibitory action (Table 2). PAC fraction showed the highest activity contribution rate, 52.4%, Among single polyphenol components, PGG showed the highest activity contribution rate, 9.3%,

3. Sucrose tolerance test on rats

(1) Influence of evening primrose extract on blood glucose level after loading sucrose (Fig. 2 A)

Values of the group that had evening primrose extract were significantly lower than the values of the group that had 0 mg/kg of the extract; 30 minutes ($P<0.05$) after the loading sucrose in the group that had 500 mg/kg of the extract, 30 minutes ($P<0.01$) and 60 minutes ($P<0.05$) after loading sucrose in the group that had 1,000 mg/kg of the extract.

(2) Influence of evening primrose extract on blood glucose level after loading glucose (Fig. 2 B)

Values of the group that had 1,000 mg/kg of evening primrose extract were significantly lower than the values of the group that had 0 mg/kg of the extract 30 minutes ($P<0.01$) and 60 minutes ($P<0.01$) after loading glucose. Values of the group that had 500 mg/kg of the extract were significantly higher than the values of the group that had 0 mg/kg of the extract 120 minutes ($P<0.05$) after loading glucose.

(3) Influence of the PAC fraction on blood glucose level after loading sucrose (Fig. 3)

Values of the group that had the PAC fraction were significantly lower than the values of the group that had 0 mg/kg of the fraction; 30 minutes ($P<0.05$) after loading sucrose in the group that had 500 mg/kg of the fraction, 30 minutes ($P<0.01$) and 60 minutes ($P<0.05$) after loading sucrose in the group that had 1,000 mg/kg of the fraction. Values of the group that had 500 mg/kg of the fraction were significantly higher than the values of the group that had 0 mg/kg of the fraction before loading sucrose ($P<0.05$). Total polyphenol content and total PAC content of the PAC fraction were 67.3% and 59.7% respectively. The percentage of total PAC in the total polyphenol content was 88.7%. The PAC fraction did not include single polyphenol components that were found in evening primrose extract in our test (PGG, gallic acid, (+)- catechin, PB1 and PB3).

(4) Influence of PGG on blood glucose level after loading sucrose (Fig. 4)

Values of the group that had PGG were significantly lower than the values of the group that had 0 mg/kg of PGG; 30 minutes ($P<0.01$) after loading sucrose in the group that had 100 mg/kg of PGG, 30 minutes ($P<0.01$) and 60 minutes ($P<0.01$) after loading sucrose in the group that had 500 mg/kg. Values of the group that had 500 mg/kg of PGG were significantly higher than the values of the group that had 0 mg/kg 120 minutes after loading sucrose ($P < 0.05$).

Table 2 α-glucosidase inhibitory action contribution rate of evening primrose extract and its polyphenol components

Component	Content in evening primrose extract (%)	IC_{50} (mg/kg)	Contribution ratio (%)
Evening primrose extract	100.0	0.34	100.0
PGG	2.7	0.10	9.3
Gallic acid	3.1	0.19	5.5
(+)- catechin	3.4	6.5	0.18
PB1+PB3	1.5 [a)]	> 2.0 [b)]	< 0.26
Total PAC	41.4	0.27 [c)]	52.4

a): Shown in a total value, because peaks of PB1 and PB3 could not be separated.

b): PB1 was used as a sample.

c): PAC fraction was used as a sample.

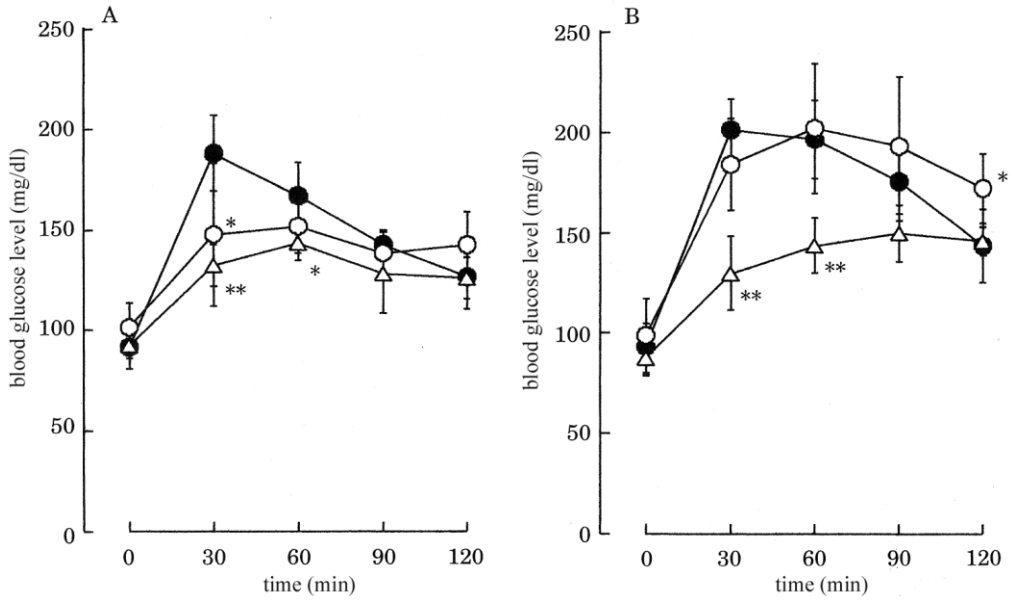

Fig. 2 Influence of evening primrose extract on blood glucose level of rats after loading sucrose (A) and glucose (B)
Average value ± standard deviation (n=6)
*: $p<0.05$ (significant difference against taking 0 mg/kg)
**: $p<0.01$ (significant difference against taking 0 mg/kg)
Administration of evening primrose extract (●, 0 mg/kg; ○, 500 mg/kg; △, 1,000 mg/kg)

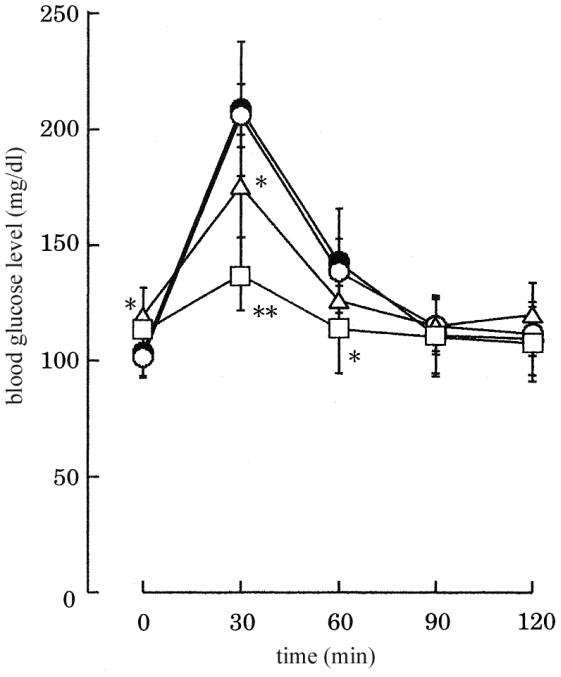

Fig. 3 Influence of the PAC fraction on blood glucose level of rats after loading sucrose
Average value ± standard deviation (n=6)
*: $p<0.05$ (significant difference against taking 0 mg/kg)
**: $p<0.01$ (significant difference against taking 0 mg/kg)
Administration of the PAC fraction (●, 0 mg/kg; ○, 100 mg/kg; △, 500 mg/kg, □, 1,000 mg/kg)

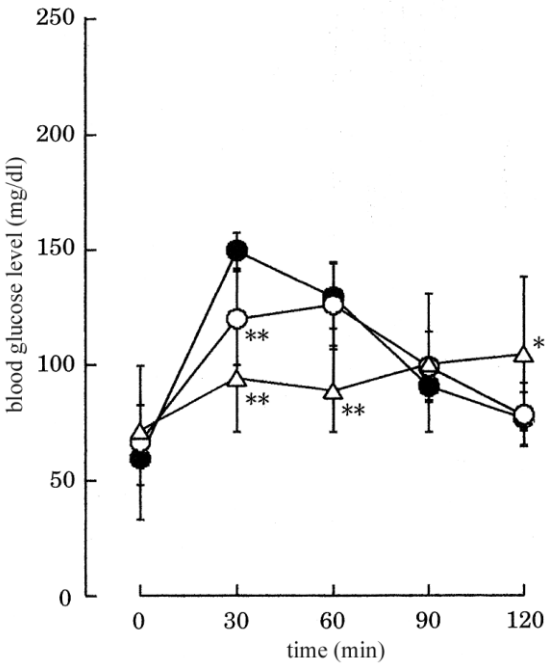

Fig. 4 Influence of PGG on blood glucose level of rats after loading sucrose
Average value ± standard deviation (n=6)
*: $p<0.05$ (significant difference against taking 0 mg/kg)
**: $p<0.01$ (significant difference against taking 0 mg/kg)
Administration of PGG (●, 0 mg/kg; ○, 100 mg/kg; △, 500 mg/kg)

Discussion

In early stages of metabolic disorder caused by diabetes, postprandial blood glucose level rises. It is believed that patients experience consistent high postprandial blood glucose level and eventually, their fasting blood glucose level becomes high as diabetes worsens[2]. Therefore, it is important to normalize the rise of postprandial blood glucose level for primary prevention of diabetes.

Some food materials that prevent the rise of postprandial blood glucose level have been discovered and most of them perform the activity by inhibiting glucose digestive enzymes[19)-21)]. Some plant-derived polyphenols are known for the inhibitory action against α-glucosidase[11)12)]. We wondered if polyphenols, contained in evening primrose extract in high concentration, would have the inhibitory action against α-glucosidase. In our tests, we aimed to clarify evening primrose extract's activity to control the rise of blood glucose level and its active components.

First, we analyzed the composition of polyphenol components contained in evening primrose extract. As a result, we confirmed that PGG, gallic acid, (+)- catechin, PB1 and PB3 exist as single polyphenol components. PAC had the highest content (41.4%). PAC is a generic term for catechin polymers and it consists of PB1, PB3, and other multi molecular species[22)]. PAC makes up over 60% of polyphenol components contained in evening primrose extract, indicating that it is the main polyphenol component in the extract.

It is known that gallic acid, (+)- catechin, and PAC, that were found in evening primrose extract in our test, are contained in various food materials[22)-24)]. Concerning PGG however, there has been almost no report on the measurement of the content in food materials though it is known that PGG exists in gallnut used in dyes and astringens[25)]. We thought that PGG might be a distinctive component of evening primrose extract since we discovered that PGG exists in the extract.

Then, we examined the activity of evening primrose extract and its polyphenol components discovered in our test to inhibit α-glucosidase in an in vitro test system. As a result, we confirmed that evening primrose extract has the inhibitory action against α-glucosidase. We also confirmed that the main activity body of the extract is PAC, main polyphenol component of the extract, since PAC's activity contribution rate to inhibit α-glucosidase was over 50%. Among single polyphenol components confirmed in our test, PGG showed the highest activity contribution rate. Other than polyohenols, several percent of protein and fat exist in the extract. Their contents are smaller than PAC content and PAC's activity contribution rate is over 50%. Taking the facts into consideration, we speculate the activity contribution rate of protein and fat are lower than the one of PAC.

Concerning food materials with the inhibitory action against α-glucosidase, there is a report about guava leaf extract. A research group lead by Ms. Deguchi orally gave mice 500 mg/kg body weigh of guava leaf extract and conducted a sucrose tolerance test. They reported that the rise of blood glucose level after loading sucrose was significantly controlled[26)]. In our sucrose tolerance test on rats as well, we confirmed that oral administration of 500 mg/kg of evening primrose extract controls the rise of blood glucose level after loading sucrose. Since we confirmed that evening primrose extract inhibits α-glucosidase in our in vitro test system, we estimate that evening primrose extract performs its activity to prevent the rise of blood glucose level by inhibiting α-glucosidase just like guava leaf extract. In our sucrose tolerance test, we gave rats evening primrose extract by different dose; 0, 500, and 1,000 mg/kg. As a result, the extract controlled the rise of blood glucose level after lading sucrose concentration-dependently. Thus, we believe that there is no problem about these concentrations for studying the effectiveness of the extract.

We believe that evening primrose extract controls the rise of blood glucose level by inhibiting α-glucosidase. Sucrose is decomposed into monosaccharide by α-glucosidase and then absorbed by the body. If evening primrose extract performs its inhibitory action against α-glucosidase in vivo, its activity to control the rise of blood glucose level performed in the sucrose tolerance test should not be performed in a glucose tolerance test. So, we conducted a glucose tolerance test on rats in order to compare the results with the sucrose tolerance test. As a result, the activity to control the rise of blood glucose level, performed in the sucrose tolerance test, was not performed in the glucose tolerance test when 500 mg/kg was used. This clarified that evening primrose extract controls the rise of blood glucose level by inhibiting α-glucosidase. However, when 1,000 mg/kg was used, the extract significantly controlled the rise of blood glucose level after lading glucose, indicating that the extract also has the activity to control glucose absorption. As an action mechanism to control glucose absorption, inhibition of glucose transporter of small intestinal epithelial cells can be pointed out. According to a report, tea polyphenol has the inhibitory action against glucose transporter of small intestinal epithelial cells[27)]. Since evening primrose extract contains high concentration of polyphenols, there is a potential that the extract has the activity to control glucose absorption via the inhibition of glucose transporter. Since evening primrose extract's activity to control glucose absorption was confirmed at a higher dose as compared to the inhibitory action against α-glucosidase, it is indicated that its activity to control the rise of blood glucose level is smaller than the inhibitory action against α-glucosidase.

We then conducted a sucrose tolerance test on rats for the PAC fraction and PGG that showed high activity contribution rate to inhibit α-glucosidase. As a result, administration of the PAC fraction and PGG significantly controlled the rise of blood glucose level after lading sucrose. This clarified that the PAC fraction and PGG have the activity to control the rise of blood glucose level just as evening primrose extract.

The minimum effective dose in the sucrose tolerance test was 500 mg/kg for evening primrose extract, 500 mg/kg for the PAC fraction, and 100 mg/kg for PGG. This indicates that the PAC fraction has as strong activity to control the rise of blood glucose level as evening primrose extract and PGG has a stronger activity than the evening primrose extract. IC_{50} value of evening primrose extract for α-glucosidase activity in vitro was 0.34 mg/ml and the values of the PAC fraction and PGG were 0.27 mg/ml and 0.10 mg/ml respectively. This indicates that the PAC fraction has as strong inhibitory action against α-glucosidase as evening primrose extract and PGG has the stronger activity than evening primrose extract. The fact that relation of strength of evening primrose extract, PAC fraction, and PGG was the same in the in vitro test for the activity to control blood glucose level and the in vitro test

for the inhibitory action against α-glucosidase supports the idea that these materials control the rise of blood glucose level by inhibiting α-glucosidase.

Since a large quantity of the PAC fraction was necessary in our in vivo test, we prepared it by scaling up the procedure to prepare the PAC fraction in our in vitro test. Due to the difference in extraction efficiency, the total polyphenol content and total PAC content of the PAC fraction used in the in vivo test were 67.3% and 59.7% respectively, lower than the PAC fraction used in the in vitro test (85.1% and 80.8%). However, these two fractions did not include single polyphenol components found in evening primrose extract in our test (PGG, gallic acid, (+)- catechin, PB1, and PB3) and the percentage of total PAC in total polyphenol was very high (around 90%). Therefore, we believe that bioactivity of polyphenols in these PAC fractions is mostly caused by PAC. Although these two fractions may have slight difference in activity strength according to extraction efficiency, we believe that there is no difference in quality because PAC is the major active component in both.

Results of the tests to specify active components of evening primrose extract in vivo indicate that PAC is the most important active component for the activity to control the rise of blood glucose level as well. Reasons are as follows; Strength of the PAC fraction's activity to control the rise of blood glucose level was almost the same as the strength of evening primrose extract. PAC had the highest content in evening primrose extract. The value was significantly higher than the content of single polyphenol components found in evening primrose extract in our test. These facts deny the possibility of polyohenol components other than PAC performs higher contribution than PAC.

The results described above suggest that evening primrose extract performs the action to control the rise of blood glucose level by inhibiting α-glucosidase and that its active component is PAC.

Summary

We conducted this study in order to clarify the activity of evening primrose extract, ethanol-extracted from defatted seeds of evening primrose (Oenothera biennis L.), to control the rise of blood glucose level and its active components.

(1) We confirmed that evening primrose extract contains of single polyphenol components; PGG 2.7%, gallic acid 3.1%, and (+)-catechin 3.4%, PB1 and PB3 1.5 % in total.

(2) Total PAC content was 41.4%.

(3) We measured these components' inhibitory action against α-glucosidase in vitro and conducted carbohydrate tolerance tests using rats. The results indicate that evening primrose extract has the activity to control the rise of blood glucose level by inhibiting α-glucosidase and that its active component is PAC.

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