Evening Primrose's Activity to Prevent the Rise of Postprandial Blood Glucose Level on People with Impaired Glucose Tolerance or Mild Symptoms of Diabetes

Crossover Test with a Placebo as Control

Oryza Oil & Fat Chemical Co., Ltd.

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[1] Purpose of the Test

Evening primrose extract's activity to inhibit disaccharidase has been clarified in former basic tests. We conducted a meal test on people with impaired glucose tolerance or mild symptoms of diabetes using a placebo as control in order to examine the extract's activity to prevent the rise of blood glucose level.

[2] Test Method

(1) Test Substance

Evening primrose extract is taken by degreasing evening primrose seed. It contains high concentration of polyphenol. We conducted a meal tolerance test using 200 mg of evening primrose extract per time. As a control, we used a placebo made of 200 mg of white rice powder. We made both test samples into dark brown capsules so they could not be differentiated from each other by their appearance, taste, or odor.

(2) Test Method

We employed a crossover meal tolerance test using a placebo as a control. We instructed the subjects not to eat or drink (except for water) since 21:00 on the previous day. They gathered at 8:00 in the morning of the test day (July 21, 2001) and rested. We then examined their physical conditions and took blood samples at 8:20. We had them intake the test samples at 8:45. We gave ten subjects capsules containing evening primrose extract and the other ten placebo capsules. Then we had them eat a meal at 8:50. All subjects ate a pack of store-bought rice (Sato no Gohan: 200 g of Koshihikari rice from Niigata Prefecture). Nutritional composition of the rice is; energy 302 kcal, protein 4.6 g, fat 1.2 g, carbohydrate 68.0 g, and Na 6 mg. We instructed the subjects to complete eating within ten minutes with 200 cc of water. We took blood samples of the subjects at 9:30 (30 min. later), 10:00 (60 min. later), 10:30 (90 min. later), and 11:00 (120 min. later) through their veins and then measured their blood glucose level, serum insulin value, and neutral fat value.

After a six-day washout period, we conducted the same test on the second test day (July 28, 2001). Test schedule was the same as the first test day except subjects took a different test sample from the previous time.
We conducted a blood test (blood biochemistry, cell components) and urine test prior to the ingestion of test samples on each day.

(3) Test Subjects
Test subjects were 18 adult persons of which fasting blood glucose level was in between 110 mg/dl and 180 mg/dl in the preliminary inspection for the test. Table 1 shows their background.

(4) Ethical Provisions
We conducted the test with the approval of the Ethics Committee of the Institute of General Medical Science (chairperson: Mr. Shoji Inoue, lawyer) following the guidelines of the Helsinki Declaration. Subjects submitted written agreement for participating in the test beforehand.

[3] Results
We crossed over the test after a one-week washout period. Table 2 shows the results of the blood biochemistry and cell components test conducted prior to the ingestion of the active sample and placebo. There was no significant difference between the two, indicating there was no difference in background of subjects before test.
Table 3 shows the change in blood glucose level after eating a meal. Before eating, blood glucose level of the subjects in the placebo group was 128.8±39.2 mg/dl. It rapidly increased after eating and became 186.4±40.2 mg/dl 30 minutes later, 225.3±52.2 mg/dl 60 minutes later, 226.9±62.3 mg/dl 90 minutes later, and 213.0±62.1 mg/dl 120 minutes later. Blood glucose level of the subjects in the active sample group was 124.7±26.1 mg/dl before eating. Increase of the level was milder as compared to the placebo group in all measurement times. Their blood glucose level was 165.5±30.4 mg/dl 30 minutes later, 209.7±40.1 mg/dl 60 minutes later, 209.1±45.9 mg/dl 90 minutes later, and 192.2±47.1 mg/dl 120 minutes later. The area below the blood glucose level rising curve after eating was significantly smaller in the placebo group as compared to the active sample group ($p<0.05$).
Fig. 1 is a graph of the variation of blood glucose level after eating. The rise of blood glucose level was always smaller in the active sample group as compared to the placebo group. The rise was significantly lower in the measurements conducted 30 and 120 minutes later.
Table 4 shows the change in insulin value after eating. There was almost no difference in the two groups before eating. Insulin secretion was low in the measurements conducted 30 and 60 minutes later in the active sample group. The area below the insulin rising curve to 120 minutes

later was 46.8±31.2 μl▪hr/dl in the placebo group. The area was 39.4±34.5 μU▪hr/dl in the active sample group. This indicates that the evening primrose extract controls the rise of insulin value even though the effect is not significant.

Table 5 shows the change in neutral fat value. There was no significant difference between the two groups.

[4] Consideration and Summary

The test results clarify that evening primrose extract prevents rapid rise of blood glucose level after eating, which helps to prevent rapid increase of insulin secretion. This indicates that evening primrose extract prevents high postprandial blood glucose level and also reduces the burden of insulin secretion on diabetes patients with insulin secretion deficiency.

Evening primrose extract's activity to inhibit saccharidase has been clarified in former basic tests on animals. The results of our test on humans support the conventional reports concerning the extract's activity and also clarify that the extract effectively works on humans. Moreover, no adverse events occurred in the test, indicating the extract is very safe when it is used in the dosage employed in the test.

The test results show that evening primrose extract stabilizes blood glucose level on diabetes patients with insulin secretion deficiency or patients with insulin resistant diabetes by preventing the rapid rise of blood glucose level caused by ingestion of meals. With the activity, the extract has a potential to prevent complications.

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Osami Kajimoto (doctor reporting the test results), Takeo Takahashi from the Institute of General Medical Science

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(3) Test Subjects
Test subjects were 18 adult persons of which fasting blood glucose level was in between 110 mg/dl and 180 mg/dl in the preliminary inspection for the test. Table 1 shows their background. There was no significant difference in blood biochemistry and cell components of subjects in the active sample group and placebo group.

(4) Ethical Provisions
We conducted the test with the approval of the Ethics Committee of the Institute of General Medical Science (chairperson: Mr. Shoji Inoue, lawyer) following the guidelines of the Helsinki Declaration. Subjects submitted written agreement for participating in the test beforehand.

Table 1 Background of Subjects (before the ingestion of the active sample)

Sex	male: 15 female: 3
Age (years old)	53.5±7.1
Height (cm)	167.6±6.3
Weight (kg)	70.9±10.3
Body mass index	25.2±2.5
Fasting blood glucose level (mg/dl)	124.7±26.1
HbA1c (%)	6.0±1.1
Total cholesterol (mg/dl)	201.4±36.1
Triglyceride (mg/dl)	198.7±101.6
Total protein (g/dl)	7.5±0.8
Persons with abnormal glucose in urine	++ 1 + 3 ±2 - 12
Systolic blood pressure (mmHg)	143.7±12.2
Diastolic blood pressure (mmHg)	88.2±14.2

[3] Results

Table 2 shows the change in blood glucose level and insulin value after eating a meal. Blood glucose level of the subjects in the placebo group rapidly increased after eating. Blood glucose level of the subjects in the active sample group was 124.7±26.1 mg/dl before eating. Increase of the level was milder as compared to the placebo group in all measurement times. Their blood glucose level was 165.5±30.4 mg/dl 30 minutes later, 209.7±40.1 mg/dl 60 minutes later, 209.1±45.9 mg/dl 90 minutes later, and 192.2±47.1 mg/dl 120 minutes later. The area below the blood glucose level rising curve after eating was significantly smaller in the placebo group as compared to the active sample group ($p<0.05$). There was almost no difference in insulin values of the two groups before eating. Insulin secretion was low in the measurements conducted 30 and 60 minutes later in the active sample group. The area below the insulin rising curve was smaller in the active sample group even though the difference was not significant.

Fig. 1 is a graph of the variation of blood glucose level after eating. The rise of blood glucose level was always smaller in the active sample group as compared to the placebo group. The rise was significantly lower in the measurements conducted 30 and 120 minutes later.

Table 2 Changes of Blood Glucose Level and Insulin Value in a Meal Tolerance Test

Group	Meal tolerance test: Blood glucose level (mg/dl)					Area below the blood glucose level rising curve (mg▪hr/dl)
	Before eating	30 min.	60 min.	90 min.	120 min.	
Active	124.726.1	165.530.4	209.740.1	209.145.9	192.247.1	123.941.3
Placebo	128.839.2	186.440.2	225.352.2	226.962.3	213.062.1	147.160.2
Significant difference between groups	n.s.	$p < 0.05$	$p < 0.1$	$p < 0.05$	$p < 0.01$	$p < 0.05$

Group	Meal tolerance test: Insulin value (μU/dl)					Area below the insulin rising curve (μU▪hr/dl)
	Before eating	30 min.	60 min.	90 min.	120 min.	
Active	12.7±9.8	22.4±14.5	35.8±33.1	43.7±35.9	42.7±31.4	39.4±34.5
Placebo	13.3±15.0	28.4±23.9	40.0±31.1	43.6±28.1	50.1±30.0	46.8±31.2
Significant difference between groups	n.s.	$p < 0.1$	n.s.	n.s.	n.s.	n.s.

paired t test

[4] Consideration and Summary

The test results clarify that evening primrose extract prevents rapid rise of blood glucose level after eating, which helps to prevent rapid increase of insulin secretion. This indicates that evening primrose extract prevents high postprandial blood glucose level and also reduces the burden of insulin secretion on diabetes patients with insulin secretion deficiency.

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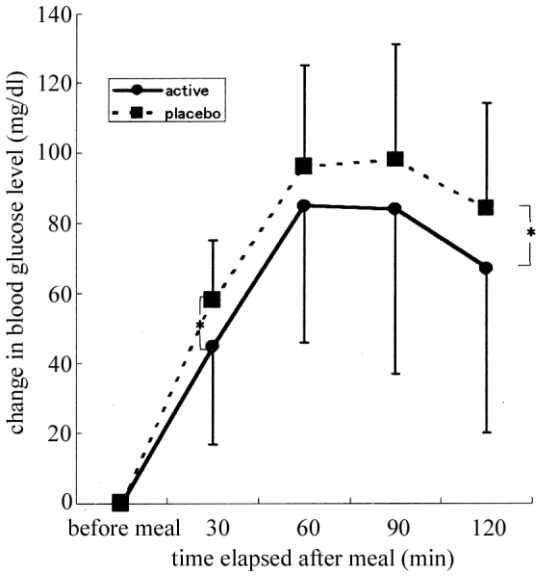

Fig. 1 Variation of Blood Glucose Level in a Meal Tolerance Test

Table 1 Background of Subjects (before the ingestion of the active sample)

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Age (years old)	53.5±7.1
Height (cm)	167.6±6.3
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Total cholesterol (mg/dl)	201.4±36.1
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Total protein (g/dl)	7.5±0.8
Persons with abnormal glucose in urine	++ 1 + 3 ±2 - 12
Systolic blood pressure (mmHg)	143.7±12.2
Diastolic blood pressure (mmHg)	88.2±14.2

Table 2 Major Blood Test Measurement Values before Eating

Subject (unit)		Group	Before Eating	Test Method
White blood cell count (/μl)	3500-9700	active placebo	6139±1180 6439±1575	Electric resistance detection method SLS-Hb method
Red blood cell count (x10000/μl)	377-577	active placebo	474±52 451±73	Red blood cell wave pulse high level detection method
Platelet count (x10000/μl)	14-38	active placebo	22.9±5.8 22.8±6.2	
Total protein (g/dl)	6.5-8.2	active placebo	7.5±0.8 7.4±0.6	Biuret method
GOT (U/l)	5-40	active placebo	33.3±23.7 33.3±20.7	UV method
GPT (U/l)	5-45	active placebo	38.5±31.0 38.0±28.0	
gamma-GTP (U/l)	0-60	active placebo	109.9±127.5 110.6±126.2	L-γ-glutamyl-3-carboxy-4-nitroanido substrate method
Total cholesterol (mg/dl)	150-220	active placebo	201.4±36.1 205.1±35.6	Enzyme method
Triglyceride (mg/dl)	50-150	active placebo	198.7±101.6 173.2±107.9	
HbA_{1c} (%)	4.3-5.8	active placebo	6.0±1.1 6.0±1.0	Latex agglutination method
Urea nitrogen (mg/dl)	8-20	active placebo	17.8±5.6 17.4±5.3	Urease UV method
Creatinine (mg/dl)	0.6-1.3	active placebo	1.5±1.8 1.5±1.6	Alkali picric acid method
Na (mEq/l)	135-145	active placebo	140.3±1.8 140.9±1.7	Electrode method
K (mEq/l)	3.5-5.0	active placebo	4.9±0.5 4.8±0.6	
Cl (mEq/l)	98-108	active placebo	102.8±2.1 102.4±1.9	

not significant

Table 3 Blood Glucose Levels in a Meal Tolerance Test (mg/dl) and the Area below the Curve

Group	Meal tolerance test: Blood glucose level (mg/dl)					Area below the blood glucose level rising curve (mg▪hr/dl)
	Before eating	30 min.	60 min.	90 min.	120 min.	
Active	124.7±26.1	165.5±30.4	209.7±40.1	209.1±45.9	193.2±47.1	123.9±41.3
Placebo	128.8±39.2	186.4±40.2	225.3±52.2	226.9±62.3	213.0±62.1	147.1±60.2
Significant difference between groups	n.s.	$p < 0.05$	$p < 0.1$	$p < 0.05$	$p < 0.01$	$p < 0.05$

paired t test

Table 4 Serum Insulin Values in a Meal Tolerance Test and the Area below the Curve

Group	Meal tolerance test: Insulin value (μU/dl)					Area below the insulin rising curve (μU▪hr/dl)
	Before eating	30 min.	60 min.	90 min.	120 min.	
Active	12.7±9.8	22.4±14.5	35.8±33.1	43.7±35.9	42.7±31.4	39.4±34.5
Placebo	13.3±15.0	28.4±23.9	40.0±31.1	43.6±28.1	50.1±30.0	46.8±31.2
Significant difference between groups	n.s.	$p < 0.1$	n.s.	n.s.	n.s.	n.s.

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Table 5 Neutral Fat Value in a Meal Tolerance Test

Group	Meal tolerance test: Neutral vat value (mg/dl)				
	Before eating	30 min.	60 min.	90 min.	120 min.
Active	199±102	275±125	333±129	356±141	340±107
Placebo	173±108	251±114	298±142	314±129	303±135
Significant difference between groups	n.s.	n.s.	n.s.	n.s.	n.s.

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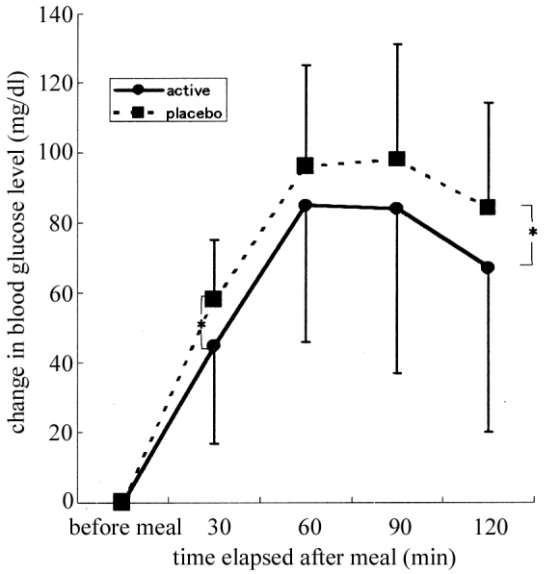

Fig. 1 Variation of Blood Glucose Level in a Meal Tolerance Test