Effects of single intake of tablets containing Evening Primrose seed extract on postprandial blood glucose levels and long-term effects on fasting blood glucose levels and safety profile of once-daily tablets

Journal of Nutritional Food Vol. 5 No. 4 2002

Takashige Koba, Koichiro Hamada, Hiroyuki Kimura, Yasuhiro Abiru, Kiyohiko Magata, Noriaki Kikuchi, Satoshi Muneyuki, and Kenji Takazawa

Oryza Oil & Fat Chemical Co., Ltd.

May 2, 2003

Effects of single intake of tablets containing Evening Primrose seed extract on postprandial blood glucose levels and long-term effects on fasting blood glucose levels and safety profile of once-daily tablets

Journal of Nutritional Food Vol. 5 No. 4 2002

Takashige Koba*, Koichiro Hamada*, Hiroyuki Kimura*, Yasuhiro Abiru*, Kiyohiko Magata*, Noriaki Kikuchi*, Satoshi Muneyuki**, and Kenji Takazawa***, ****

We had subjects take tablets containing evening primrose extract once a day for 12 weeks continuously and conducted a meal tolerance test (MTT) using boiled rice at the beginning and the end of the study period in order to examine the influence of the tablets on postprandial blood glucose level and fasting glucose level during the test period. We also examined the safety of intake of the tablets containing evening primrose extract for 12 weeks. The subjects were 44 males, mainly with impaired glucose tolerance (35 with impaired glucose tolerance (IGT), and 9 with type 2 diabetes). The test was conducted by a placebo-controlled double-blind method.

On the first day of the test period, we gave subjects a meal tolerance test using boiled rice. In subjects with IGT, the rise in postprandial blood glucose level in response to the MTT was reduced by taking the tablets containing evening primrose extract. This action was confirmed after continuous intake of the tablets for 12 weeks as well. After taking the tablets for 12 weeks, fasting blood glucose levels were significantly lowered in the treated IGT subjects compared to the baseline ($P<0.05$). Fasting blood glucose levels of the treated IGT subjects (treatment group) were significantly lower than the levels of those in controls (control group) four weeks later ($P<0.05$) and the levels of the treatment group were lower than the levels of the control group eight weeks later ($P=0.05$). HbA_{1c} value was also lowered after continuous intake of the tablets just like fasting blood glucose levels. During the entire ingestion period, there was no adverse reaction.

These results suggest that the tablet containing evening primrose seed extract has an action to prevent the rise of postprandial blood glucose levels and control blood glucose levels of people with IGT. They also suggest that the tablet is a very safe, useful supplement.

Keywords: Evening primrose seed extract, postprandial blood glucose, fasting blood glucose, impaired glucose tolerance

1. Introduction

Diabetes's typical symptom is high blood glucose level as a result of insulin shortage or impaired insulin action[1]. Symptoms develop gradually. In early stages, high blood glucose level is shown only after a meal has been taken. It becomes consistent and eventually, fasting blood glucose level becomes high as diabetes worsens[2]. Therefore, it is important to prevent the rise of postprandial blood glucose level to prevent the progression of diabetes and complications. In clinical aspect, alpha-glucosidase inhibitor that controls the decomposition of disaccharide into monosaccharide was released in the 1990's in Japan[3)4)]. Some food components with saccharide digestion inhibitory activity have been discovered and some of them have been approved as food for specified health use[5)-8)]. We studied evening primrose extract with high concentration of polyphenols taken from seed as a new food material to prevent the rise of postprandial blood glucose level.

Evening primrose is a plant that belongs to oenothera, onagraceae. Pickled or boiled root is used to an appetite stimulant[9]. The Black Foot, a native American tribe, boiled the leaves to eat and dried the root to make preservatives[9]. Recently, it has been discovered that evening primrose oil extracted from seed contains a large amount of gamma-linolenic acid and therefore, the oil is effective to combat obesity, diabetes, and premenstrual syndrome. Although there have been a number of studies about oils and fats included in seed, there were almost no studies on other components of evening primrose. The research group lead by Aitani has indicated that evening primrose extract with high polyphenol content has gallic acid, catechin, pentagalloyl glucose, and proanthocyanidin[10]. Evening primrose extract has actions to inhibit actions of alpha-glucosidase and alpha-amylase. Its activity to prevent the rise of blood glucose level has been reported as a result of meal tolerance tests on animals using starch and simple sugar[10]. There is also a report of evening primrose's effect on human to prevent the rise of blood glucose level after eating rice[10].

* Saga Nutraceuticals Research Institute, Otsuka Pharmaceutical Co., Ltd.
5006-5 Aza Higashiyama, Oaza Omagari, Higashi Sekishin-son, Kanzaki-gun, Saga Prefecture 842-0195
** Consumer Products Development Department, Otsuka Pharmaceutical Co., Ltd.
3-2-27 Otedori, Chuo-ku, Osaka City, Osaka Prefecture 540-0021
*** Waseda Clinic 3-10-21 Takada Toshima-ku, Tokyo 171-0033
**** Internal Medicine Department II, Tokyo Medical College 6-7-1 Nishi-shinjuku, Shinjuku-ku, Tokyo 160-0023

In order to prevent the rise of postprandial blood glucose level, food with an action to prevent it needs to be taken with meals. After consideration about portability and convenience for users, we concluded that tablets would be the best and developed a tablet containing evening primrose extract. We decided to examine the effect of taking the tablet once a day because taking it with every meal might be too much of burden for general consumers.

We conducted a test where subjects, mainly ones with impaired glucose tolerance, took tablets containing evening primrose once a day for 12 weeks. We conducted a meal tolerance test using boiled rice at the beginning and the end of the study period in order to examine the influence of the tablets on postprandial blood glucose level and fasting glucose level during the test period. We also examined the safety of ingestion of the tablets containing evening primrose for 12 weeks.

Subjects that met the conditions above were 44 persons (all subjects) and they were all male. Table 1 shows their background. The test controller separated them into two groups; 23 subjects to take tablets containing evening primrose extract (treatment group) and 21 subjects to take control food (control group) by their age and fasting blood glucose level. Nine of the 44 subjects had fasting blood glucose level of 126 mg/dl or higher. We categorized them as diabetes patients and the other 35 subjects as ones with impaired glucose tolerance according to the advice of the diagnostic criteria examination committee of the Japan Diabetes Society[11].

We conducted the test with the approval of Waseda Clinic's clinical research screening committee following the guidelines of the Helsinki Declaration. Subjects received sufficient explanation about the test contents and methods from a doctor in advance and gave written consent to participate in the test.

Table 1. Background of Subjects

	Treatment Group	Control Group
Number of subjects	23	21
Age (year)	44.0 ± 11.3	44.6 ± 9.5
Hight (cm)	169.1 ± 4.3	172.8 ± 8.1
Weight (kg)	78.4 ± 11.4	81.4 ± 10.7
BMI	27.4 ± 4.0	27.3 ± 3.2
Fasting glucose level (mg/dl)	113 ± 14	113 ± 11

2) Test Food

For the test food containing evening primrose, we used a round tablet which contains 50 mg of evening primrose. For the evening primrose extract contained in the test food, we used a material ethanol-extracted from evening primrose (Oenothera biennis) seed that contains at least 60% polyphenols (product name: Evening Primrose Extract-P manufactured by Oryza Oil & Fat Chemical Co., Ltd.) For the control food, we used a tablet with a diluent instead of evening primrose extract. We colored the control food's surface with caramel so it could not be differentiated from the test food by appearance. Prior to the test, we had the subjects with impaired glucose tolerance take one tablet containing evening primrose extract (50 mg as evening primrose extract), two tablets (100 mg as the extract), and six tablets (300 mg as the extract) and conducted a meal tolerance test using boiled rice with comparing with placebo. As a result, the rise of postprandial blood glucose level was prevented when six tablets were taken. In the food test therefore, we had the subjects take six tablets containing evening primrose extract per time.

2. Test Method

1) Subjects

Test subjects were adults aged between 20 and 64 without any history of diabetes treatment within a month before the test that satisfied either of the following conditions.

i) Fasting blood glucose level is at least 110 mg/dl and HbA_{1c} value is under 6.5%.

ii) Two-hour value of 75 g glucose tolerance test (OGTT) is at least 140 mg/dl but under 200 mg/dl.

iii) Normal type* yet one-hour value of OGTT is at least 180 mg/dl. (* Normal type: fasting blood glucose level is under 110 mg/dl and two-hour value of OGTT is under 140 mg/dl)

3) Test Schedule

The test was conducted by a placebo-controlled double-blind method.

Test period included one week observation period prior to the ingestion of the extract and 12 weeks ingestion period. We conducted a physical examination, hematological test, blood biochemical test, and urine test on the first day of the observation period (before the ingestion), and 4, 8, and 12 weeks after starting the ingestion. We also conducted a meal tolerance test using boiled rice on the first day and 12 weeks after starting the ingestion. During the 12 week ingestion period, subjects took 6 tablets containing evening primrose extract or control product right before dinner daily. We instructed them to avoid overeating and overdrinking and spend their everyday life regularly.

We had the subjects to write a daily journal during the test period in order to learn about their physical conditions.

4) Measurement Method

a) Boiled rice tolerance test

We conducted a meal tolerance test using boiled rice with doctor's supervision on the first day and 12 weeks after starting the ingestion of the test foods. Subjects stayed in Waseda Clinic where the test was conducted from afternoon of the day before the meal test, had a specified dinner (same meal for the day before starting the ingestion of the test food and 12 weeks later: boiled rice, sweet-sour pork, bean sprout salad, and bean-starch vermicelli soup: calorie 836 kcal, protein 33.6 g, fat 19.5 g, carbohydrate 126.8 g, water 418.5 g, ash 5.9 g, dietary fiber 5.5 g) by 21:00. They fasted until the test on the following day. On the following morning, we took subjects' blood and gave them six tablets containing evening primrose extract or a control product with 100 ml water. Five minutes later, the subjects had 200 g boiled rice with 2.5 g seasoned powder ("Sato no Gohan" produced by Sato Foods Industries Co., Ltd.: calorie 302 kcal, protein 4.6 g, fat 1.2 g, carbohydrate 68 g, sodium 6 mg) in ten minutes. We took the subjects' blood 30, 60, 90, and 120 minutes after eating the boiled rice while the subjects kept rested on a bed. We used the blood samples to measure blood glucose level. We asked Mitsubishi Chemical BCL Corporation to measure blood glucose level.

b) Physical examination, hematological test, blood biochemical test, and urine test

We conducted physical examination, hematological test, blood biochemical test, and urine test prior to the ingestion of the test food and 4, 8, and 12 weeks after starting the ingestion with doctor's supervision. Measurement items were as follows. Physical examination (weight, systolic blood pressure, diastolic blood pressure, pulse rate); hematological test (white blood cell count, red blood cell count, hematocrit value, platelet count, hemoglobin content, whole blood density); blood biochemical test (fasting blood glucose level. HbA_{1c}, fructosamine, insulin, C-peptide, total cholesterol, HDL-cholesterol, LDL-cholesterol, neutral fat, free fatty acid, amylase, total protein, albumin, A/G ratio, total bilirubin, direct bilirubin, ALP, LDH, CPK, uric acid, BUN, creatinine, GOT, GPT, gamma-GTP, serum iron, TIBC, Na, K, Ca, Mg, Cl); urine test (pH, protein, sugar, urobilinogen, bilirubin, ketone body, occult blood, density). We took the subjects' blood after fasting (except for water) since 21:00 on the previous day. We conducted physical examination and urine test at Waseda Clinic and contracted out the measurement of each item for hematological and blood biochemical tests to Mitsubishi Chemical BCL Corporation.

5) Statistics Analysis

All measurement values are indicated by average value±standard deviation. We employed t-test for calibration of variation on a same subject. For comparison between groups, we basically employed Student t-test. However, when uneven variance was confirmed by homoscedasticity test based on F-test, we employed Aspin-Welch t-test. For both cases, we employed two-tail test and significance level of 5 percent at the maximum.

We evaluated all cases and subjects with impaired glucose tolerance for the variation in blood glucose level after eating boiled rice at the beginning and 12 weeks later and also the variation in blood glucose-related test values during the ingestion period. We did not evaluate these variations on diabetes patients because the number of the subjects was small (treatment group: 5, control group: 4). We evaluated safety on all subjects.

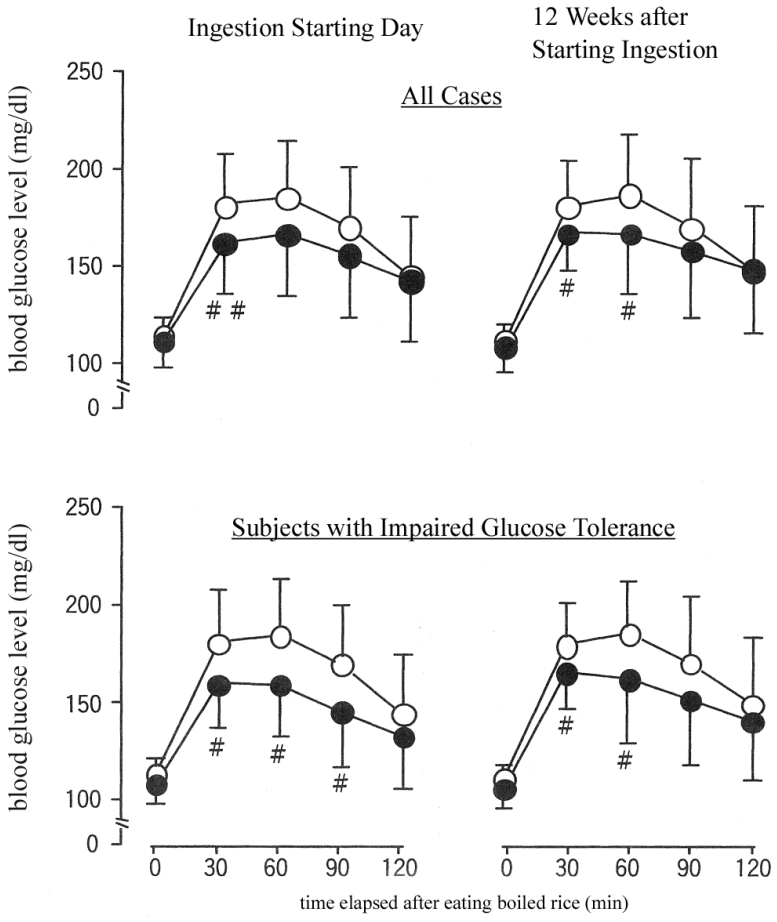
3. Results

1) Variation in blood glucose level after eating boiled rice on the first day of the ingestion period of test foods

The left panel of Fig. 1 shows the variation in blood glucose level after eating boiled rice on the first day of the ingestion period of test foods.

a) Analysis on all cases

In the comparison of blood glucose level on each specified time



●: Treatment Group ○: Control Group
Average SD #: $p<0.05$ significant difference against the control group

Fig. 1 Influence of tablets containing evening primrose extract on blood glucose level after eating boiled rice

during the boiled rice tolerance test conducted on the first day of the ingestion period, the value of the control group 30 minutes after eating boiled rice was 181±27 mg/dl and the value of the treatment group was 162±26 mg/dl. The value of the control group 60 minutes later was 185±29 mg/dl and the value of treatment group was 166±31 mg/dl. Values of the treatment group were significantly lower than the ones of the control group. ($p<0.05$)

b) Analysis on subjects with impaired glucose tolerance

In the comparison of blood glucose level of the subjects with impaired glucose tolerance on each specified time during the boiled rice tolerance test conducted on the first day of the ingestion period, the value of the control group 30 minutes after eating boiled rice was 180±27 mg/dl and the value of the treatment group was 159±22 mg/dl. The value of the control group 60 minutes later was 184±28 mg/dl and the value of treatment group was 159±26 mg/dl. The value of the control group 90 minutes later was 169±31 mg/dl and the value of treatment group was 145±28 mg/dl. Values of the treatment group were significantly lower than the ones of the control group. ($p<0.05$)

2) Variation in blood glucose level after eating boiled rice 12 weeks after starting the ingestion of test foods

The right panel of Fig. 1 shows the variation in blood glucose level after eating boiled rice 12 weeks after starting the ingestion of test foods.

a) Analysis on all cases

We compared blood glucose level on each specified time during the boiled rice tolerance test conducted 12 weeks after the starting of the ingestion of the test foods between two groups. Results were similar to the variation in blood glucose level during the boiled rice tolerance test conducted on the starting day. Blood glucose level of the control group 30 minutes after eating boiled rice was 181±23 mg/dl and the value of the treatment group was 167±18 mg/dl. The value of the control group 60 minutes later was 187±31 mg/dl and the value of treatment group was 167±31 mg/dl. Values of the treatment group were significantly lower than the ones of the control group. ($p<0.05$)

b) Analysis on subjects with impaired glucose tolerance

In the comparison of blood glucose level of the subjects with impaired glucose tolerance on each specified time during the boiled rice tolerance test, the value of the control group 30 minutes after eating boiled rice was 179±22 mg/dl and the value of the treatment group was 165±18 mg/dl. The value of the control group 60 minutes later was 185±27 mg/dl and the value of treatment group was 162±33 mg/dl. Values of the treatment group were significantly lower than the ones of the control group. ($p<0.05$)

Table 2 Variation in Blood Glucose-related Indexes

		All Cases		Subjects with Impaired Glucose Tolerance	
		Treatment Group	Control Group	Treatment Group	Control Group
Fasting blood glucose level	before injestion	113±14	113±11	108±10	110±10
	4 weeks later	105±11#**	112±9	103±9#*	112±10
	8 weeks later	106±15**	115±19	104±12*	116±21
	12 weeks later	106±13**	108±8*	104±11*	108±8
HbA1c (%)	before injestion	5.4±0.5	5.4±0.4	5.3±0.4	5.5±0.4
	4 weeks later	5.4±0.4	5.5±0.4	5.2±0.3#	5.5±0.4
	8 weeks later	5.4±0.4	5.5±0.4	5.2±0.3#*	5.5±0.3
	12 weeks later	5.3±0.5*	5.4±0.3	5.2±0.4#*	5.5±0.3
Fructosamine (μmol/l)	before injestion	253±21	249±17	249±21	248±19
	4 weeks later	249±26	250±18	244±20	251±17
	8 weeks later	257±35	257±21	249±28	257±23
	12 weeks later	235±22***	232±17***	231±20***	230±17***
Insulin (μU/ml)	before injestion	11.3±6.8	11.8±6.4	11.5±7.4	10.8±5.9
	4 weeks later	10.3±5.5	16.0±19.8	10.8±5.9	16.9±21.9
	8 weeks later	10.6±8.0	15.1±12.6	11.4±8.2	16.1±13.9
	12 weeks later	9.7±7.0	10.3±5.1	10.4±7.7	10.4±5.6
C-peptide (ng/ml)	before injestion	2.8±1.0	2.8±0.8	2.8±1.1	2.8±0.8
	4 weeks later	2.7±0.7	3.3±1.7	2.7±0.8	3.5±1.8
	8 weeks later	2.7±1.1	3.3±1.4	2.8±1.2	3.4±1.5
	12 weeks later	2.7±0.9	2.9±1.0	2.8±1.0	3.0±1.0

Average value±standard deviation
#: $p<0.05$ Significant difference against the control group
*: $p<0.05$ Significant difference against values before ingestion
**: $p<0.01$ Significant difference against values before ingestion
***: $p<0.001$ Significant difference against values before ingestion

Table 3 Variation in Physical Examination Values

		before Ingestion	4 Weeks Later	8 Weeks Later	12 Weeks Later
Weight (kg)	Treatment Group	78.4 ± 11.4	78.1 ± 11.3	78.0 ± 11.2	76.7 ± 11.0**
	Control Group	81.4 ± 10.7	81.4 ± 10.6	81.1 ± 10.6	80.1 ± 10.4***
Systolic Blood Pressure (mmHg)	Treatment Group	126 ± 12	124 ± 17	123 ± 12	118 ± 16**
	Control Group	129 ± 9	129 ± 13	128 ± 12	125 ± 9
Diastolic Blood Pressure (mmHg)	Treatment Group	75 ± 11	74 ± 12	71 ± 11*	70 ± 12**
	Control Group	78 ± 10	77 ± 10	78 ± 11	75 ± 9
Pulse Rate (/min.)	Treatment Group	72 ± 13	70 ± 13	67 ± 8**	65 ± 9***
	Control Group	71 ± 11	71 ± 10	72 ± 11	65 ± 10***

Average value ± standard deviation
*: $p<0.05$ Significant difference against values before ingestion
**: $p<0.01$ Significant difference against values before ingestion
***: $p<0.001$ Significant difference against values before ingestion

Table 4 Variation in Hematological Test Values

	Standard Value		before Ingestrion	4 Weeks Later	8 Weeks Later	12 Weeks Later
White Blood Cell Count (/μl)	3300—9000	Treatment Group	6304 ± 1521	6396 ± 1735	6265 ± 1628	6591 ± 1512
		Control Group	6224 ± 1642	6200 ± 1363	6095 ± 1211	6262 ± 1298
Red Blood Cell Count (x 10^4/μl)	430—570	Treatment Group	505 ± 47	490 ± 47**	488 ± 46**	483 ± 46***
		Control Group	514 ± 31	504 ± 34	501 ± 32*	491 ± 38***
Hemoglobin Content (g/dl)	13.5—17.5	Treatment Group	15.6 ± 0.9	15.2 ± 1.0**	15.2 ± 0.9**	14.9 ± 0.9***
		Control Group	16.0 ± 1.1	15.7 ± 0.9	15.6 ± 1.1*	15.3 ± 1.1***
Hematocrit Value (%)	39.7—52.4	Treatment Group	45.9 ± 2.8	44.5 ± 2.9**	44.1 ± 2.8***	43.9 ± 2.9***
		Control Group	47.0 ± 2.3	46.0 ± 2.7	45.5 ± 2.7**	44.9 ± 2.7***
Platelet Count (x 10^4/μl)	14.0—34.0	Treatment Group	24.1 ± 6.9	24.1 ± 5.6	23.5 ± 5.5	24.3 ± 5.8
		Control Group	23.8 ± 4.7	23.0 ± 5.9	22.3 ± 4.9**	22.9 ± 5.0*
Whole Blood Density	1.055—1.063	Treatment Group	1.057 ± 0.002	1.056 ± 0.002**	1.056 ± 0.002***	1.056 ± 0.002**
		Control Group	1.058 ± 0.002	1.057 ± 0.002	1.057 ± 0.002**	1.057 ± 0.002***

Average value ± standard deviation
*: $p<0.05$ Significant difference against values before ingestion
**: $p<0.01$ Significant difference against values before ingestion
***: $p<0.001$ Significant difference against values before ingestion

3) Variation in blood glucose-related indexes after ingesting the test foods for 12 weeks

Table 2 shows the variation in fasting blood glucose level and HbA_{1c}, fructosamine, insulin, and C-peptide values after ingesting the test foods for 12 weeks.

a) Analysis on all cases

Fasting blood glucose level of all subjects in the treatment group significantly lowered 4 weeks after starting the ingestion of the test food. The level remained significantly low 8 and 12 weeks later as well ($p<0.01$). Fasting blood glucose level of the control group did not change until 8 weeks after starting the ingestion of the test food. However, it significantly lowered 12 weeks later ($p<0.05$). In the comparison of fasting blood glucose level on each specified time, the value of the treatment group was significantly lower than the one of the control group 4 weeks later ($p<0.05$).

HbA_{1c} value of all subjects in the treatment group did not change very much until 8 weeks after starting the ingestion of the test foods. However, it significantly lowered 12 weeks later ($p<0.05$). The value of the control group did not change even 12 weeks later. There was no significant difference between two groups concerning HbA_{1c} value in each specified time.

Fructosamine value of all subjects did not change very much in either group until 8 weeks after starting the ingestion of the test foods. However, it significantly lowered 12 weeks later ($p<0.001$). There was no significant difference between two groups concerning the fructosamine value in each specified time.

Insulin and C-peptide values of all subjects did not change very much in either group throughout the ingestion period. There was no significant difference between two groups concerning values in each specified time.

b) Analysis on subjects with impaired glucose tolerance

Fasting blood glucose level of the subjects with impaired glucose tolerance of the treatment group significantly lowered 4 weeks after starting the ingestion of the test food ($p<0.05$). The level remained significantly low 8 and 12 weeks later as well (both $p<0.05$). Fasting blood glucose level of the control group did not change throughout the ingestion period. In the comparison of fasting blood glucose level on each specified time, the value of the treatment group was significantly lower than the one of the control group 4 weeks later ($p<0.05$) and the value of the treatment group tended to be lower than the one of the control group 8 weeks later ($p=0.05$).

HbA_{1c} value of the subjects with impaired glucose tolerance of the treatment group significantly lowered 8 weeks after starting the ingestion of the test foods ($p<0.05$). The level remained significantly low 12 weeks later as well ($p<0.05$). The value of the control group did not change throughout the ingestion period. In the comparison of HbA_{1c} value on each specified time, the value

Table 5 Variation in Blood Biochemical Teat Values

	Standard Value		before Ingestion	4 Weeks Later	8 Weeks Later	12 Weeks Later
Total Cholesterol (mg/dl)	120—219	Treatment Group	215 ± 35	205 ± 32*	210 ± 29	200 ± 30***
		Control Group	210 ± 29	205 ± 32	205 ± 29	198 ± 27**
HDL-Cholesterol (mg/dl)	40—70	Treatment Group	48 ± 10	46 ± 9*	45 ± 11*	43 ± 10***
		Control Group	44 ± 8	46 ± 9*	45 ± 8	40 ± 6**
LDL-Cholesterol (mg/dl)	65—139	Treatment Group	136 ± 37	136 ± 35	142 ± 34*	128 ± 32*
		Control Group	134 ± 28	140 ± 32	137 ± 27	128 ± 28
Neutral Fat (mg/dl)	30—149	Treatment Group	180 ± 103	192 ± 151	212 ± 310	176 ± 80
		Control Group	208 ± 136	167 ± 90	160 ± 74	184 ± 73
Free Fatty Acid (mEq/l)	0.1—0.9	Treatment Group	0.5 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
		Control Group	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
Total Protein (g/dl)	6.2—8.9	Treatment Group	7.4 ± 0.3	7.2 ± 0.3**	7.2 ± 0.3**	6.8 ± 0.3***
		Control Group	7.5 ± 0.3	7.2 ± 0.4***	7.2 ± 0.3***	6.8 ± 0.4***
Alubumin (g/dl)	3.8—5.3	Treatment Group	4.6 ± 0.2	4.6 ± 0.2	4.6 ± 0.2	4.3 ± 0.2***
		Control Group	4.7 ± 0.2	4.6 ± 0.2**	4.6 ± 0.2*	4.4 ± 0.2***
A/G Ratio	1.1—2.2	Treatment Group	1.7 ± 0.1	1.7 ± 0.2*	1.8 ± 0.2***	1.8 ± 0.2***
		Control Group	1.7 ± 0.1	1.8 ± 0.1***	1.8 ± 0.2***	1.8 ± 0.2***
Total Bilirubin (mg/dl)	0.2—1.7	Treatment Group	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.3	0.7 ± 0.3
		Control Group	0.7 ± 0.3	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.2
Direct Billirubin (mg/dl)	0.2—0.8	Treatment Group	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	0.4 ± 0.2
		Control Group	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
ALP (IU/l/37℃)	100—325	Treatment Group	234 ± 44	230 ± 54	230 ± 52	209 ± 40***
		Control Group	206 ± 51	210 ± 55	210 ± 53	190 ± 49*
LDH (IU/l/37℃)	120—240	Treatment Group	190 ± 40	180 ± 34*	184 ± 37	167 ± 37***
		Control Group	171 ± 26	168 ± 28	170 ± 27	157 ± 27***
CPK (IU/l/37℃)	60—270	Treatment Group	184 ± 108	162 ± 64	185 ± 142	173 ± 139
		Control Group	138 ± 48	148 ± 66	153 ± 107	138 ± 77
GOT (IU/l/37℃)	10—40	Treatment Group	27 ± 8	25 ± 9	26 ± 10	22 ± 8***
		Control Group	23 ± 8	23 ± 9	24 ± 10	23 ± 12
GPT (IU/l/37℃)	5 —45	Treatment Group	44 ± 23	39 ± 22	37 ± 21*	34 ± 20**
		Control Group	37 ± 23	38 ± 26	40 ± 30	39 ± 30
γ-GPT (IU/l/37℃)	≦80	Treatment Group	62 ± 46	55 ± 36*	56 ± 41	50 ± 35*
		Control Group	54 ± 19	56 ± 27	58 ± 28	59 ± 27
Amylase (IU/l/37℃)	55—175	Treatment Group	81 ± 22	81 ± 23	78 ± 22	78 ± 22
		Control Group	78 ± 19	78 ± 20	91 ± 60	77 ± 33
Uric Acid (mg/dl)	3.8—7.5	Treatment Group	6.7 ± 1.3	6.5 ± 1.1	6.7 ± 1.3	6.4 ± 1.1*
		Control Group	6.0 ± 1.3	6.1 ± 1.3	6.1 ± 1.4	6.2 ± 1.5
BUN (mg/dl)	8 —23	Treatment Group	14 ± 3	15 ± 4	14 ± 3	14 ± 3
		Control Group	13 ± 2	13 ± 3	14 ± 3	14 ± 3
Creatinine (mg/dl)	0.8—1.3	Treatment Group	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
		Control Group	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
Na (mEq/l)	137—147	Treatment Group	141 ± 2	140 ± 1	140 ± 1	140 ± 1
		Control Group	140 ± 2	140 ± 1	140 ± 1	140 ± 1*
K (mEq/l)	3.5—5.0	Treatment Group	4.1 ± 0.3	4.3 ± 0.2**	4.2 ± 0.3	4.2 ± 0.3
		Control Group	4.2 ± 0.2	4.3 ± 0.2	4.1 ± 0.3	4.2 ± 0.2
Ca (mg/l)	8.4—10.4	Treatment Group	9.5 ± 0.3	9.5 ± 0.3	9.7 ± 0.3*	9.2 ± 0.4**
		Control Group	9.6 ± 0.3	9.5 ± 0.3	9.6 ± 0.2	9.2 ± 0.3***
Mg (mg/l)	1.9—2.5	Treatment Group	2.3 ± 0.2	2.2 ± 0.1*	2.2 ± 0.3*	2.2 ± 0.1*
		Control Group	2.3 ± 0.1	2.2 ± 0.2*	2.2 ± 0.3	2.2 ± 0.2
Cl (mEq/ml)	98—108	Treatment Group	102 ± 2	102 ± 2	102 ± 2	102 ± 2
		Control Group	102 ± 2	102 ± 2	102 ± 2	103 ± 2*
Serun Iron (μg/dl)	50—200	Treatment Group	105 ± 33	103 ± 46	100 ± 28	106 ± 37
		Control Group	131 ± 48	113 ± 37	117 ± 56	123 ± 44
TIBC (μg/dl)	270—425	Treatment Group	343 ± 34	336 ± 36*	340 ± 35	321 ± 32***
		Control Group	331 ± 22	324 ± 29	325 ± 24	308 ± 27***

Average value +/- standard deviation
*: $p<0.05$ Significant difference against values before ingestion
**: $p<0.01$ Significant difference against values before ingestion
***: $p<0.001$ Significant difference against values before ingestion

of the treatment group was significantly lower than the one of the control group 4 weeks later ($p<0.05$), 8 weeks later ($p<0.05$), and 12 weeks later ($p<0.05$).
Fructosamine value of the subjects with impaired glucose tolerance did not change very much in either group until 8 weeks after starting the ingestion of the test foods. However, it significantly lowered 12 weeks later ($p<0.001$). There was no significant difference between two groups concerning fructosamine value in each specified time.
Insulin and C-peptide values of the subjects with impaired glucose tolerance did not change very much in either group throughout the ingestion period. There was no significant difference between two groups concerning values in each specified time.

4) Variation in values in physical examination after the 12-week ingestion of test foods
Table 3 shows the variation in the subjects' weight, blood pressure, and pulse rate after ingesting the test foods for 12 weeks. Weight of subjects in both groups significantly lowered 12 weeks later (treatment group: $p<0.01$, control group $p<0.001$). Systolic blood pressure of the subjects in the treatment group significantly lowered 12 weeks later ($p<0.01$) and the value of the subjects in the control group tended to lower ($p<0.10$). Similarly, diastolic blood pressure of the subjects in the treatment group significantly lowered 12 weeks later ($p<0.01$) and the value of the subjects in the control group tended to lower ($p<0.05$). Pulse rate of the subjects in both groups significantly lowered 12 weeks later ($p<0.001$). These variations in physical examination values have no problem in safety.

5) Variation in clinical test values after the 12-week ingestion of the test food
Tables 4 and 5 show the variation in hematological test and blood biochemical test values after the 12-week ingestion of the test foods. In the hematological test, red blood cell count, hemoglobin content, hematocrit value, and whole blood density significantly lowered 12 weeks later in both groups ($p<0.001$, whole blood density of the treatment group: $p<0.01$). Platelet count of the control group significantly lowered 12 weeks later ($p<0.05$). These variations are within the standard range and there is no problem in safety. In the blood biochemical test, total cholesterol, HDL-cholesterol, total protein, albumin, A/G ratio, ALP, LDH, Ca, and TIBC values significantly lowered 12 weeks later ($p<0.001$, Ca value of the treatment group, total cholesterol and HDL-cholesterol value of the control group: $p<0.01$, ALP value of the control group: $p<0.05$) in both groups. LDL-cholesterol value of the treatment group significantly lowered 12 weeks later ($p<0.05$) and the value of the control group tended to lower ($p=0.06$). GOT ($p<0.001$), GPT ($p<0.01$), gamma-GTP ($p<0.05$), uric acid ($p<0.05$), and Mg values ($p<0.05$) significantly lowered only in the treatment group. These variations are within the standard range and there is no problem in safety. There was no variation that could indicate a problem in safety in the measurement items of urine test in either party.

6) Adverse effects undeniably related to the 12-week ingestion of the test foods
Some adverse effects undeniably related to the ingestion of the tablets containing evening primrose extract or control food occurred in the 12-week ingestion period. In the treatment group, seven diarrhea cases, one fatigue case, and one palpitation case were reported. In the control group, there was a case that exceeded the upper limit of the CPK standard range and also a case that exceeded the upper limit of the uric acid standard range. Eight diarrhea cases, one fatigue case, one excessive sweating case, two headache cases, one stomachache case, and one abdominal bloating sensation case were reported. These adverse effects occurred sporadically and temporarily and symptoms were mild so we concluded that there is no problem in safety of the use of the extract.

4. Discussion

According to the "Diabetes Field Survey"[12] conducted by the Ministry of Health and Welfare (currently MHLW) in November 1997, there were 6,900,000 people with strong suspicion of diabetes (including people receiving medical treatment) in Japan. This survey indicated that 13,700,000 people were diabetic including ones with undeniable potential of diabetes. It is expected that diabetes patients will keep increasing. In early stages of metabolic disorder caused by diabetes, postprandial blood glucose level rises. As diabetes worsens, high postprandial blood glucose level becomes consistent and eventually, fasting blood glucose level rises[2]. Therefore, it is important to prevent a rise in postprandial blood glucose level to prevent the progression of diabetes and complications.
In order to prevent the rise of postprandial blood glucose level, pharmaceuticals such as alpha-glucosidase inhibitor have been developed[13]. Lately, a number of studies have been conducted about food materials for this purpose and some food components with the activity to prevent the rise of postprandial blood glucose level have been discovered[5)-8)]. We studied evening primrose extract with high concentration of polyphenols taken from seed as a new food material to prevent the rise of postprandial blood glucose level. Evening primrose extract's activity to inhibit alpha-glucosidase and alpha-amylase has been confirmed in vitro[10]. A research group lead by Mr. Aitani had healthy people take evening primrose extract one time and conducted a boiled rice tolerance test five minutes later. As a result, they confirmed that the extract prevented a rise in blood glucose level[10]. In our test conducted on people with impaired glucose tolerance, we confirmed that the rise of blood glucose level after eating boiled rice was lower in the group that took evening primrose extract as compared to the control group. This effect was the same after the 12-week continuous ingestion of the extract, indicating that the extract is an effective food material to prevent the rise of postprandial blood glucose level for people with impaired glucose tolerance.
It is believed that prevention of the rise of postprandial blood glucose level for each meal is more effective to prevent diabetes. A group lead by Mr. Nakamura has reported that taking alpha-glucosidase inhibitor once a day significantly lowers blood glucose level[14], indicating that alpha-glucosidase inhibitor does not necessarily have to be taken at each meal. Development of an effective food product, that can perform its function when it is taken once a day, is desirable for users' convenience. In our test, we discovered that taking tablets containing evening primrose extract at dinner for 12 weeks lowered fasting blood glucose level of people with impaired glucose tolerance. In a comparison test, we confirmed that fasting blood glucose level of the subjects

significantly lowered 4 weeks later and tended to be low 8 weeks later as compared to the level of the control group. HbA_{1c} value also lowered after continuous ingestion of the tablets just like fasting blood glucose level. Similar to Mr. Nakamura's report[14], these results indicate that the extract can control blood glucose level just by daily ingestion. We believe that continuous daily intake of the tablet with a meal will help to control blood glucose level of people with impaired glucose tolerance.

In our test, weight of the subjects in both groups reduced by approximately 2 percent in 12 weeks. We believe that this is because the subjects wrote a daily journal during the test period and the consciousness of being a test subject helped them to improve their lifestyle. To back up our speculation, health parameters such as serum lipid level and liver function also improved 12 weeks later in both groups. Blood pressure level of the subjects in the treatment group significantly lowered and the level of the subjects in the control group tended to lower as well.

In our test, fructosamine value of all subjects in both groups lowered 12 weeks after starting the ingestion of the test foods. Sugar in blood bonds with protein with free amino group and forms stabilized ketoamine, namely fructosamine. Most of fructosamine derives albumin. Serum half-life of albumin is approximately two weeks. Frucrosamine value varies in proportion to the blood glucose level that frucrosamine is exposed to during the two weeks[15]. We believe that frucrosamine value of the control group lowered because blood glucose level, total protein, and albumin lowered 12 weeks later. On the other hand, HbA_{1c} value lowered only in the subjects in the treatment group in our test. We assume this is because HbA_{1c} value reflects blood glucose control condition in past one to three months[16]. We gather that blood glucose level, total protein, and albumin values lowered 12 weeks later because subjects' lifestyle was improved.

During out test, seven subjects in the treatment group and eight subjects in the control group reported diarrhea as subjective symptom. As abdominal symptom of alpha-glucosidase inhibitor (medicinal drug), feeling of fullness, increased farts, and diarrhea have been reported[3)4)]. It is believed that feeling of fullness and increased farts are caused because organic acids such as acetic acid, butyric acid, and lactic acid are produced when undigested carbohydrate is decomposed by intestinal germs in the large intestine[17)18)]. Diarrhea is caused by the rise of osmotic pressure when organic acids increase in intestines[18)]. In our test, subjects did not experience any adverse event such as abdominal swelling due to the ingestion of the test food. We do not think that diarrhea was caused by evening primrose extract's alpha-glucosidase inhibitory activity because the diarrhea frequency in the treatment group was almost the same as the control group. In the test where subjects ingested test foods every day for 12 weeks, diarrhea cases occurred singly or sporadicly. Therefore, we assume diarrhea was most likely caused by subject-related factors such as diet.

There is a concern that the ingestion of alpha-glucosidase inhibitor may cause low blood glucose level[3)4)]. However, there was no such case in the long-period ingestion of tablets containing evening primrose extract.

Evening primrose extract includes high concentration of polyphenol. Polyphenol strongly inhibits iron absorption. It is known that this occurs due to catechol group which is a gallic acid group of polyphenolic compound[19)20)]. There was a worry that long-period ingestion of evening primrose extract may cause iron deficiency due to iron absorption inhibition. In our test, continuous ingestion of tablets containing evening primrose extract did not lower serum iron. TIBC and hemoglobin content significantly lowered in the treatment group. However, they lowered in the control group as well and the variation was within the normal range. There was no individual case that serum iron, TIBC, or hemoglobin content went beyond the normal range throughout the test period. Thus, 12-week continuous ingestion of six tablets did not cause iron deficiency.

Since there was no adverse event to question the safety of the tablet containing evening primrose extract when the tablet was ingested continuously for 12 weeks, we consider that the tablet is very safe food.

Based on the test results described above, we conclude that the tablet containing evening primrose has the activity to prevent the rise of postprandial blood glucose level and control blood glucose level of people with impaired glucose tolerance when it is taken daily continuously. The tablet is considered to be a very safe, useful food.

5. Conclusions

We had test subjects, mainly ones with impaired glucose tolerance, ingest tablets containing evening primrose extract continuously for 12 weeks. We conducted a meal tolerance test using boiled rice on the beginning and the end of the test period in order to examine the influence of the tablets on blood glucose level and fasting glucose level during the test period. We also examined the safety of ingestion of the tablets for 12 weeks. The results were as follows:

1) On a meal tolerance test using boiled rice on the beginning of the ingestion period, ingestion of the tablets containing evening primrose extract controlled the rise of blood glucose level of people with impaired glucose tolerance after eating boiled rice. This effect was the same after the 12-week continuous ingestion of the tablets.

2) Taking the tablets containing evening primrose extract at dinner for 12 weeks lowered fasting blood glucose level of people with impaired glucose tolerance. Fasting blood glucose levels of the subjects in the treatment group 4 and 8 weeks later were lower than the levels of the subjects in the control group. HbA_{1c} value also lowered after continuous ingestion of the tablets just like fasting blood glucose level.

3) There was no adverse event to question the safety of the tablet containing evening primrose extract when the tablet was ingested continuously for 12 weeks

Based on the results described above, we conclude that the tablet containing evening primrose has the activity to prevent the rise of postprandial blood glucose level and control blood glucose level of people with impaired glucose tolerance when it is taken daily continuously. The tablet is considered to be a very safe, useful food.

Reference Literature

1) Goto Y, Baba S, Nakagawa S, Kuzutani K, Hirata Y, Akanuma Y, Sakamoto N, Shigeta Y, Tarui S, Kaneko T, Mimura G: Helpfulness of alpha-glucosidase inhibitor AO-128 on noninsulin-dependent diabetes, Third phase double blind comparison test between two groups. *Igaku no Ayumi*, **160** (12),

943-971, 1992.
2) Iwamoto Y: Control of postprandial blood glucose level. *Nihon Rinsho*, **55**, special issue Diabetes (2), 721-726, 1997.
Pharmaceuticals 2002. 2022-2023, *Jiho*, 2002.
5) Deguchi Y, Nagata K, Naganami O, Kobayashi K, Ohashi A, Tachikawa T, Watanuki M, Oka M, Nakajima K, Hata N: Helpfulness and safety of continuous/excessive ingestion of hot water-extracted Guava leaf. *J. Jap. Soc. New Food Ingredients*, **3** (1), 19-28, 2000.
6) Morimoto S, Takana S, Miyazaki T, Kodama T, Kitamura I, Inoue S: Effects and safety of long-term ingestion of wheat-derived albumin on mild NIDDM patients. *Jap. Soc. Nutrition and Food Science's Journal*, **52** (5), 293-300, 1999.
7) Nukii K, Tanaka Y, Seri K, Inoue S: Variation of blood glucose level after the ingestion of table sugar with L-arabinose on healthy humans. *J. Nutritional Food*, **4** (1), 13-18, 2001.
8) Fujita H, Yamagami T, Ohshima K: Long-term ingestion of a fermented soybean-derived Touchi-extract with alpha-glucosidase inhibitory activity is safe and effective in humans with borderline and mild type-2 diabetes. *J. Nutr*., **131** (8), 2105-2108, 2001.
9) Study on functions of medicinal herbs. 29-33, Health Industry Newspaper, 1999.
10) Aitani A, Yamaguchi H: Functions and applications of polyphenols derived from evening primrose seed. *FRAGRANCE JOURNAL*, **29** (5), 98-103, 2001.
11) Kuzutani K, Nakagawa S, Sato Y, Kanazawa Y, Iwamoto Y, Kobayashi T, Nanjo K, Sasaki Y, Kiyono H, Ito C, Shima K, Nonaka K, Kadowaki T: Commission report on the classification of diabetes and diagnostic criteria. *Diabetes*, **42** (5), 385-401, 1999.
12) Lifestyle-related Diseases Management Office, Health Service
3) Japan Pharmaceutical Information Center: Japanese Pharmaceuticals 2002. 3-5, *Jiho*, 2002
4) Japan Pharmaceutical Information Center: Japanese
Bureau, Ministry of Health and Welfare: Diabetes Field Survey. 1999.
13) Goto Y: Glucosidase inhibitor. *Diabetes Frontier*, **3** (6), 557-564, 1992
14) Nakamura T, Sasaki I, Masada M, Tamai T, Sasada K, Nakai T: Influence of daily administration of alpha-glucosidase inhibitor on blood glucose level. *Hospital Pharmacy*, **24** (5), 473-478, 1998.
15) Kawanishi K, Ishida T, Kajikawa T, Tada T: Fructosamine. *Nihon Rinsho*, **53**, special issue, Wide range blood/urine chemical tests and immunologic tests (1), 546-548, 1995.
16) Kawahara R: Glycol-hemoglobin. *Nihon Rinsho*, **53**, special issue, Wide range blood/urine chemical tests and immunologic tests (1), 543-545, 1995.
17) Maruhama Y, Nagasaki A, Kanazawa Y, Hirakawa H, Goto Y, Nishiyama H, Kishimoto Y, Shimoyama T: Effects of a glucoside-hydrolase inhibitor (BAY g 5421) on serum lipids, lipoproteins and bile acids, fecal fat and bacterial flora, and intestinal gas production in hyperlipidemic patients. *Tohoku J. Exp. Med*., **132** (4), 453-462, 1980
18) Creutzfeldt W: Proceedings, First international symposium on Acarbose. Amsterdam, Excerpta Medica ICS 594, 1982.
19) Bravo L: Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.*, **56** (11), 317-333, 1998.
20) Brune M, Rossander L, Hallberg L: Iron absorption and phenolic compounds; Importance of different phenolic structures. *Eur. J. Clin. Nutr.*, **43** (8), 547-558, 1989.

Received on November 6, 2002.