Antidiabetic Food

Caiapo

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1. Introduction

The International Diabetes Federation (IDF) has reported that the number of diabetic patients in the world will increase to 380 million, and the prevalence of the disease will reach 7% of all adults by 2025. The number of patients in China, where the economy is growing remarkably, has increased particularly rapidly, to 92.4 million, which is the greatest number in any country, and exceeds the 40.9 million patients in India.

Currently, these are the problems not only in developed countries, but also in developing countries. Therefore, preventing the onset and/or development of diabetes is a major challenge in terms of medical expenses.

Since diabetes is asymptomatic and left untreated in many cases, it may result in a chronic state of hyperglycemia, which leads to the development of complications such as arteriosclerosis, retinopathy, and nephropathy. The real concern with diabetes is not in the disease itself, but in a significant decrease in quality of life (QOL) due to complications resulting from the disease.

Although the mechanism of diabetes has not yet been elucidated, it is thought to be a multifactorial disease caused by an interaction among various factors such as heredity and lifestyle (e.g., dietary habits and lack of activity). In particular, the most important risk factors are these related to the dietary habits, such as overeating and high-fat dietary intake, as suggested by the fact that the dietary therapy is used as the first step of diabetes treatment. For this reason, to keep potential diabetic patients from becoming real diabetic patients, it would be effective to mitigate hyperglycemic effects by improving dietary habits.

Given this, there is a great need to develop food products that may be of help in mitigating hyperglycemic effects in order to prevent the development of diabetes.

As part of our research into the physiological functions of foods, we discovered that a variety of white ocarinas called Caiapo potato (registered trade name: Caiapo) has curative effects against insulin resistance that may also be effective against hyperglycemia in diabetic patients. Several foods for specified health use have been developed for the treatment of hyperglycemia with the main effect of sugar absorption in the gastrointestinal tract. Additionally, Caiapo is an innovative food product, because it has curative properties against insulin resistance. Accordingly, Caiapo is expected to improve the dietary habits of diabetic patients and have hypoglycemic effects enabling more effective glycemic control over diet.

Caiapo is a variety of *Ipomoea batatas* (sweet potato) native to Latin America. Caiapo is a food with a proven safety profile, because it has been eaten by the local people for a long time.

2. Diabetes

2-1. Causes

Diabetes is a metabolic disorder caused by decline in function of insulin (insulin resistance) or reduced secretion of insulin, resulting in chronic hyperglycemic symptoms. Specifically, hyperglycemia is maintained by decreased sugar uptake to the muscle tissue and/or increased carbohydrate synthesis (enhanced gluconeogenesis) in the liver. Although the mechanism of diabetes has not yet been elucidated, it is thought to be a multifactorial disease caused by an interaction among various factors such as heredity and lifestyle (e.g., dietary habits and lack of activity).

In recent years, it has been suggested that the inflammatory state of adipose tissue is deeply involved in the development of insulin resistance that triggers hyperglycemia.

Sustained hyperglycemia due to diabetes can cause vascular and neurological disorders leading to various complications, such as retinopathy, neuropathy, ischemic heart disease, arteriosclerosis, and obesity, which may result in more serious conditions.

2-2. Classification

Diabetes can broadly be classified into type 1 and type 2 diabetes (abstract from *Diabetes Mellitus*, **53**, 450-467, 2010).

Type 1 Diabetes: This type develops as insulin depletes due to a destructive lesion of pancreatic β cells caused by autoimmunity. In addition to genetic factors, some triggers and/or environmental factors, such as viral infection, are also involved in the development of diabetes. Characteristically, most of the patients are tented to have an absolute deficiency of insulin. And it rapidly develops mostly in young people, but it can develop in all age groups. Patients with type 1 diabetes make up only approximately 5% of all diabetic patients.

Type 2 Diabetes: This type develops as insulin activity is decreased by several genetic factors that cause impaired insulin secretion or insulin resistance, as well as by environmental factors related to lifestyle, such as overeating, lack of activity, and obesity. Unlike type 1 diabetes, it rarely requires insulin injection, because the pancreatic β-cell function is maintained to a certain degree, and it develops in and after middle age in most cases. Most diabetic patients (approx. 95%) have this type of diabetes.

2-3. Treatments

In general, dietary/exercise therapies are used as the first step in the treatment of diabetes, however, if it is determined that blood sugar is not controlled sufficiently with these therapies, medications are to be used in combination with them.

Medications used include oral hypoglycemic drugs and insulin injections.

As shown in **Table 1**, oral hypoglycemic drugs are classified into the following three categories: insulin secretagogues, postprandial hyperglycemia improving drugs, and insulin resistance improving drugs.

Table 1. Characteristics of Oral Hypoglycemic Drugs

Category	Action System/Mechanism		Type	Drug Name	Adverse Drug Reactions
Insulin secretagogues	Pancreatic islet	Promotion of insulin secretion	Sulfonylurea (SU drug)	Glimepiride, Glibenclamide, Gliclazide, Tolbutamide	Hypoglycemia
		More rapid promotion of insulin secretion	Glinids (Rapid-acting insulin secretagogues)	Nateglinide, Mitiglinide	
Postprandial hyperglycemia improving drugs	Small intestine	Retardation of carbohydrate absorption Improvement of postprandial hyperglycemia	Alpha-glucosidase inhibitor	Voglibose, Acarbose, Miglitol	Liver disorder, Gastrointestinal symptoms (Flatus, diarrhea, abdominal distension, constipation)
	Small intestine	Inhibition of carbohydrate absorption	Biguanides	Metformin	Lactic acidosis
Insulin resistance improving drugs	Liver	Inhibition of gluconeogenesis Improvement of insulin resistance		Buformin	Gastrointestinal disorders, Enhanced hypoglycemia
	Fat	Improvement of insulin resistance	Thiazolidins	Pioglitazone	Edema/cardiac failure, Liver disorder/ weight gain, Enhanced hypoglycemia

The use of these drugs (medication only or combination with dietary/exercise therapy) will be determined in consideration of their characteristics and adverse reactions according to the symptoms of each patient (pathology/disposition). Insulin injection will be used for patients who have severe adverse reactions or insufficient control of blood sugar during the treatment with the above-mentioned medications.

In addition, a drug with a novel mechanism of action called dipeptidyl peptidase 4 (DPP-4) inhibitor was developed, and there is currently great interest in it. By inhibiting the effect of an enzyme (DPP-4) that breaks down incretin, a gastrointestinal hormone that promotes insulin secretion in a glucose-dependent manner, DPP-4 inhibitor raises blood incretin levels. These results suggest that this incretin (GLP-1: glucagon-like peptide 1) does not cause hypoglycemia, because it only works in the event of hyperglycemia. In addition, it is expected that DPP-4 inhibitor not only acts on pancreatic β-cells, but is also effective in retarding the elimination of food from the stomach and suppressing appetite/obesity.

3. Effects of Caiapo

Caiapo has been tested for efficacy not only in animals (spontaneously diabetic mice, etc.) but also in humans. The results demonstrated that Caiapo is effective in treating diabetes.

The test results are shown as follows.

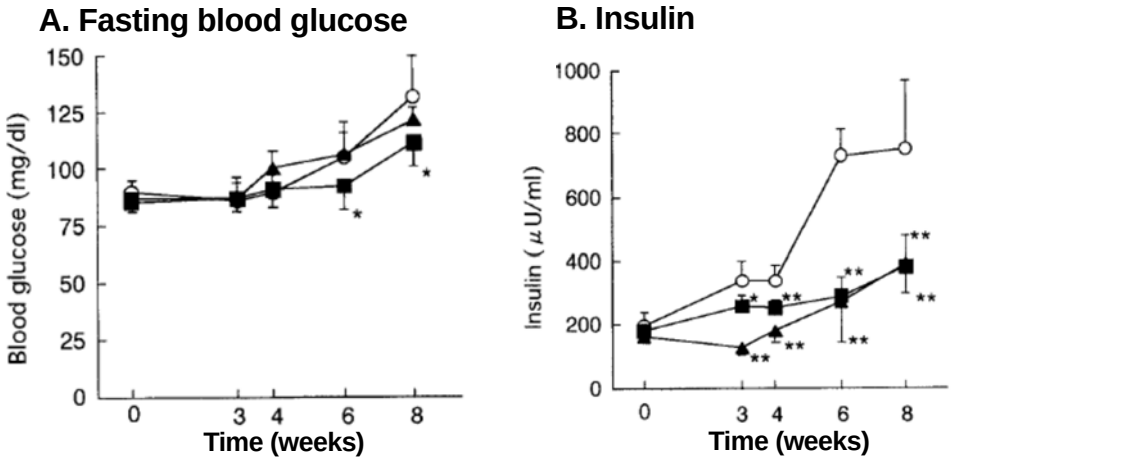
3-1. Animal Tests

3-1-1. Effects on Fasting Blood Glucose Levels (Zucker *fa/fa* rats) [1)]

<Continuous Administration Test>

An 8-week continuous administration test was performed on diabetic model Zucker *fa/fa* rats using an internal dialysis solution powder of Caiapo (100 mg/kg/day).

The results demonstrated that, in the Caiapo group, the fasting blood glucose level was significantly decreased at Weeks 6 and 8 after administration ($p < 0.05$ for both, **Figure 1A**). In addition, the insulin level was significantly decreased at Weeks 3, 4, 6, and 8 showing that hyperinsulinemia was improved ($p < 0.01$ for both, **Figure 1B**).



○: Control; ■: Caiapo (100 mg/kg); ▲: Troglitazone (50 mg/kg)

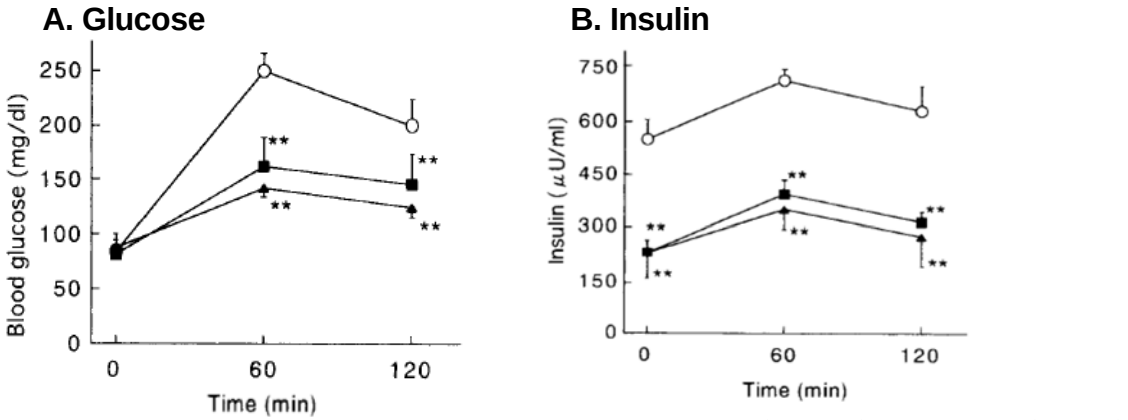
Mean±SD (n=7): * $p < 0.05$, ** $p < 0.01$ vs. Control group

Figure 1 Effects of Caiapo on Fasting Blood Glucose Levels and Insulin Levels

The results suggested that the Caiapo administration is effective in improving insulin resistance.

<Oral Glucose Tolerance Test (OGTT)>

The results of an oral glucose tolerance test (amount of glucose load: 2 g/kg) performed at Week 7 demonstrated that, in comparison with the control group, the increase in blood glucose and insulin secretion after loading were significantly suppressed in the Caiapo group (**Figures 2A** and **2B**, respectively).



○: Control; ■: Caiapo (100 mg/kg); ▲: Troglitazone (50 mg/kg)

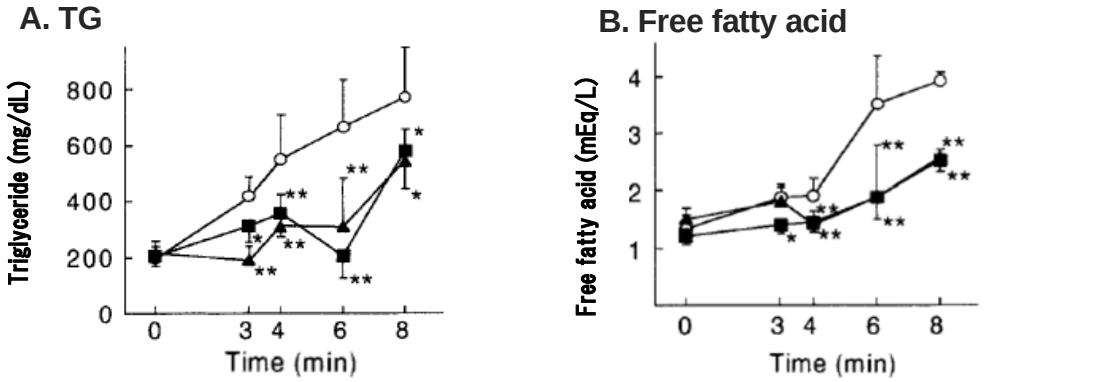
Mean±SD (n=7): ** $p < 0.01$ *vs.* Control group

Figure 2 OGTT after Continuous Administration of Caiapo

These results suggests that, because the insulin secretion was suppressed as the increase in blood glucose was suppressed, the administration of Caiapo improved insulin sensitivity resulting in a decrease in fasting blood glucose level and a suppression of blood sugar level increase.

<Effects on Blood Lipid Levels>

Triglyceride (TG) and free fatty acid levels were measured in order to determine the effects of Caiapo on blood lipid levels (**Figures 3A** and **3B**, respectively).



○: Control; ■: Caiapo (100 mg/kg); ▲: Troglitazone (50 mg/kg)

Mean ± SD (n = 7): * $p < 0.05$, ** $p < 0.01$ *vs.* Control Group

Figure 3 Effects of Caiapo on Blood Lipid Levels

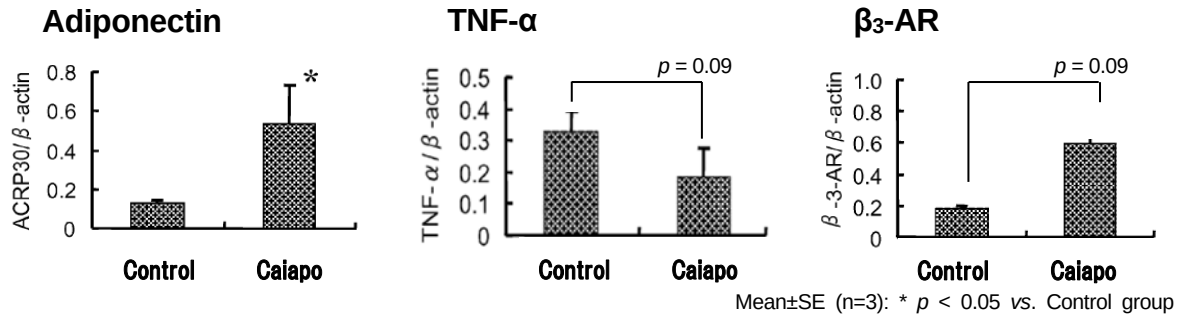
In the Caiapo group, TG and free fatty acid levels decreased continuously from Week 3 to Week 8. Thus, it suggests that the Caiapo administration is also effective in decreasing blood lipid levels.

In conclusion, it was confirmed that Caiapo ingestion suppresses hyperglycemia by reducing insulin resistance. This study also suggested that Caiapo is effective in suppressing increases in blood lipids (TG and free fatty acids).

3-1-2. Mechanism of Action (*KKA*^{y} mouse) [2)]

To examine the mechanism of hypoglycemic action of Caiapo, a continuous administration test was performed in *KKA*^{y} mice. After completing the administration, adipose tissue was extracted to examine the effects of administration on the expression level of adipocytokine.

As shown in **Figure 4**, the results of expression level measurement using the PCR method (internal standard: β-Actin) demonstrated that, in comparison with the control group, the expression level in the Caiapo group was significantly increased for adiponectin ($p < 0.05$), had a decreasing tendency for tumor necrosis factor-α (TNF-α) ($p = 0.09$), and had an increasing tendency for β_3-adrenergic receptor (β_3-AR) ($p = 0.09$).



Mean±SE (n=3): * $p < 0.05$ *vs*. Control group

Figure 4 Measurement of the Expression in Adipose Tissue

It has been demonstrated that adipose tissues function as energy storage, but also play an important role in controlling glucose/lipid metabolism by secreting various adipocytokines. In particular, adiponectin works as an insulin-sensitivity enhancing factor, and it is known that adiponectin decreases under diabetic conditions[3)]. Additionally, it is proved that TNF-α increases under diabetic conditions, and works as an insulin tolerance precipitating factor[4)]. These adipocytokines suppress expression in adipose tissues, and, in this study, it is demonstrated that the expression level after the administration of Caiapo was significantly increased for adiponectin ($p < 0.05$) but had a decreasing tendency for TNF-α ($p = 0.09$). Adiponectin is also known to increase energy consumption, in which β_3-AR and uncoupling protein (UCP-1) are involved in thermogenesis[5)], and it has been shown that their expression levels have an increasing tendency with the administration of Caiapo.

In conclusion, the results of the present study suggested that the administration of Caiapo promotes energy consumption through the improvement of abnormal adipocytokine secretion, and exerts its hypoglycemic effect by suppression of insulin resistance.

3-2. Active Components

3-2-1. Structure of Arabinogalactan-protein Isolated from Caiapo (Caiapo-derived AGP) [6)]

Various candidate compounds from Caiapo were isolated and purified in order to elucidate its major active components involved in hypoglycemic activity. In doing so, the hyperglycemia

suppression effect was used as an index. An arabinogalactan-protein (hereafter "Caiapo-derived AGP") was isolated from Caiapo as a major active component.

Caiapo-derived AGP is a glucoprotein with a molecular weight of approximately 130,000 Da, and consists of protein (5-10%) and carbohydrate (90-95%).

As shown in **Table 2**, the protein portion consists of 17 amino acids, including the major components of hydroxyproline (24.5 mol%), alanine (18.2 mol%), and serine (13.7 mol%).

Table 2. Amino Acid Composition in the Protein moiety of Caiapo-derived AGP

	Amino Acid	Composition Ratio (mol%)		Amino Acid	Composition Ratio (mol%)
1	Aspartic acid	5.6	10	Leucine	2.8
2	Threonine	8.4	11	Tyrosine	0.6
3	Serine	13.7	12	Phenylalanine	1.1
4	Glutamic acid	5.1	13	Lysine	2.8
5	Glycine	5.1	14	Histidine	0.9
6	Alanine	18.2	15	Arginine	0.6
7	Valine	5.0	16	Hydroxyproline	24.5
8	Methionine	0.6	17	Proline	2.8
9	Isoleucine	2.2		Total	100.0

The polysaccharide moiety mainly consists of α-L-Rha, α-L-Ara, β-D-Gal, and β-D-GlcA (molar ratio = 1.0 : 4.1 : 7.6 : 1.3) with the main chain consisting of the partial structure **A**: (1→3)-β-D-galactan and the side chain connecting to the C-6 position. The side chain consists of the partial structure **B**: (1→6)-β-D-galactan with the partial structures **C** and **D** connecting to the C-3 position and the partial structure **E** connecting to the C-6 position (**Figure 5**, patent applications 2006-512842 and 2011-183349).

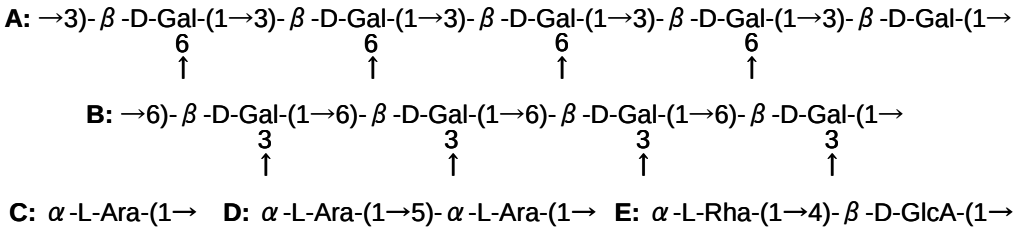


Figure 5 Structure of Caiapo-derived AGP (Carbohydrate moiety)

3-2-2. Effects of the Active Components (*db/db* mice) [7)]

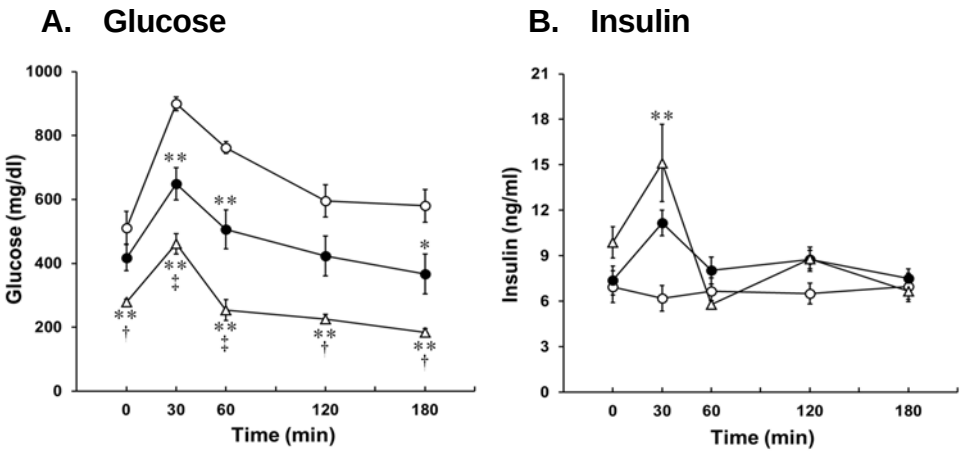
<Oral Glucose Tolerance Test (OGTT)>

A continuous administration test was conducted using gavage administration to spontaneously diabetic animal models (*db/db* mice), to examine the antidiabetic effect of Caiapo-derived AGP.

Specifically, an OGTT (glucose: 1 g/kg) was carried out at Week 6 in the control group (purified water), Caiapo-derived AGP group (20 mg/kg), and pioglitazone group (20 mg/kg) as a positive control.

The results are shown in **Figures 6A** (changes in blood glucose level) and **6B** (changes in insulin

level).



○: Control Group; ●: Caiapo-derived AGP Group（20 mg/kg）; △: Pioglitazone Group（20 mg/kg）

Mean ± SE (n=6): * $p < 0.05$,** $p < 0.01$ *vs*. Control Group; † $p < 0.05$, ‡ $p < 0.01$ *vs*. Caiapo-derived AGP Group

Figure 6　OGTT after Administration of Caiapo-derived AGP

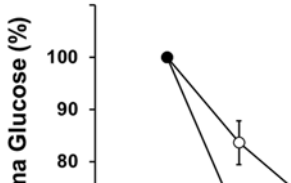
The results demonstrated that the blood glucose levels at 30, 60, and 180 minutes after loading were significantly lower in the Caiapo-derived AGP group than those in the control group (**Figure 6A**). No significant difference was observed in changes in insulin level, but insulin levels were higher in the Caiapo-derived AGP group (**Figure 6B**). In *db/db* mice, hyperinsulinemia occurs in the early stage (younger age in weeks), but insulin secretion decreases with pancreatic insufficiency as they grow older.

In conclusion, these results suggest that Caiapo-derived AGP has a pancreas protecting action, because insulin levels were higher in the Caiapo-derived AGP group, showing that the insulin secretory capacity was maintained.

<Insulin Tolerance Test (ITT)>

As with the above mentioned test, an insulin loading test (2 Units/kg) was conducted in *db/db* mice after 6 weeks of continuous administration.

The result of the ITT was shown in **Figure 7**.



○: Control Group; ●: Caiapo-derived AGP Group (20 mg/kg)

Mean±SE (n=6):* $p < 0.05$ *vs*. Control Group

Figure 7 ITT after Administration of Caiapo-derived AGP

The result demonstrated that, in comparison with the control group, the blood glucose level in the Caiapo-derived AGP group significantly and continuously decreased from 30 to 120 minutes after loading ($p < 0.05$ for both).

This suggests that Caiapo-derived AGP contributed to the improvement of insulin sensitivity and decrease in blood glucose level.

<Blood Chemistry>

After the OGTT and a 2-week administration period (at Week 8), blood tests were performed in all subjects.

The results of blood chemistry were shown in **Table 3**.

Table 3 Blood Chemistry at Week 8 after Administration of Caiapo-derived AGP

		Control Group	Caiapo-derived	Pioglitazone
Glucose	(mg/dL)	804.4 ± 78.7	504.9 ± 36.2 *	408.4 ± 78.8 **
Insulin	(ng/mL)	4.80 ± 0.89	7.87 ± 1.13	6.27 ± 0.93
TG	(mg/dL)	170.2 ± 43.4	208.0 ± 20.6	96.3 ± 14.8 †
NEFA	(mEq/L)	1.58 ± 0.11	1.47 ± 0.07	0.88 ± 0.07 ** ‡
Leptin	(ng/mL)	71.0 ± 9.6	76.3 ± 13.4	126.2 ± 7.6 ** †
Adiponectin	(μg/mL)	20.4 ± 1.6	20.3 ± 1.7	50.6 ± 3.7 ** ‡
hs-CRP	(ng/mL)	6.04 ± 0.21	5.26 ± 0.10 *	5.29 ± 0.24

Mean±SE (n=6): * $p < 0.05$, ** $p < 0.01$ *vs*. Control Group; † $p < 0.05$, ‡ $p < 0.01$ *vs*. Caiapo-derived AGP Group

The fasting blood glucose level was significantly lower in the Caiapo-derived AGP group than in the control group ($p < 0.05$). No significant difference was observed at Week 6, but a significant difference was observed after continuous administration (at Week 8). These results are similar to the results from a human clinical trial which shall be mentioned below (**3-3-2**).

In addition, the results demonstrated that the high-sensitive C-reactive protein (hs-CRP) level was significantly lower in the Caiapo-derived AGP group than in the control group ($p < 0.05$). Since hs-CRP is correlated with inflammatory cytokines such as interleukin-6 (IL-6) and TNF-α[8-10], these results suggest that the secretion of inflammatory cytokines may be suppressed by the administration of Caiapo-derived AGP.

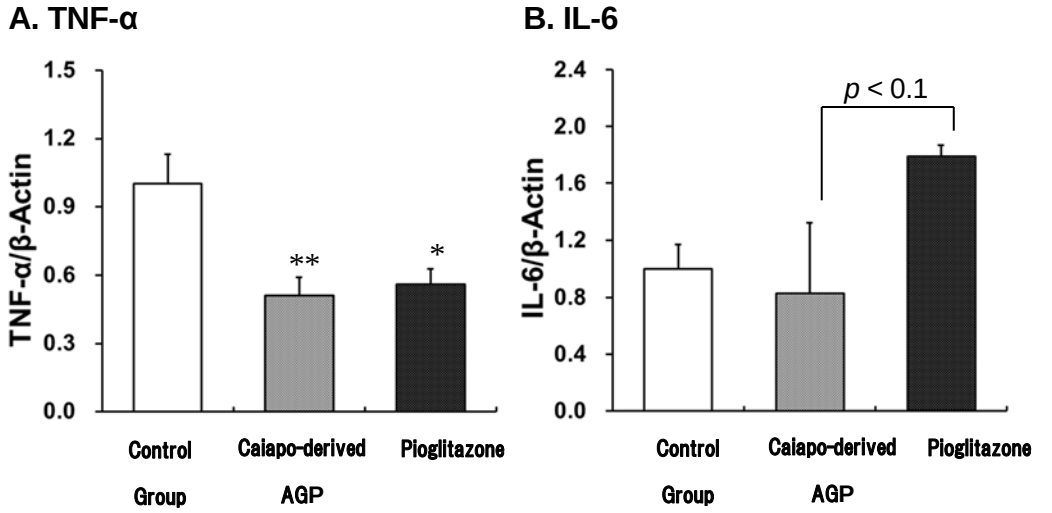
3-2-3. Mechanism of Action (*db/db* mice) [7]

<Expression Analysis of Adipose Tissue (Effects on Inflammatory Cytokines)>

Insulin resistance is one of the major factors involved in hyperglycemia, and, in recent years, it has been suggested that the inflammatory condition of adipose tissue is closely related to the development of insulin resistance. Many studies revealed it that inflammatory cytokines, IL-6 and TNF-α, interfered with the action of insulin[8-10].

Furthermore, a gene expression analysis was performed on the mesenteric fat extracted after the completion of this study to investigate the effects on inflammatory cytokines.

The results demonstrated that, in comparison with the control group, significantly decreased expression of TNF-α was observed in the Caiapo-derived AGP group (control group: 1.00±0.13 *vs*. Caiapo-derived AGP group: 0.51±0.08; $p < 0.01$; **Figure 8A**); whereas no difference was observed in the expression of IL-6 (**Figure 8B**)



Mean±SE (n=6): * $p < 0.05$, ** $p < 0.01$ *vs*. Control Group

Figure 8 Expression Analysis of Inflammatory Cytokines in Adipose tissue

In conclusion, these results suggest that the involvement of Caiapo-derived AGP in the anti-inflammatory effect is one of the mechanisms leading to the improvement of insulin resistance.

3-3. Examination in Human Clinical Trials

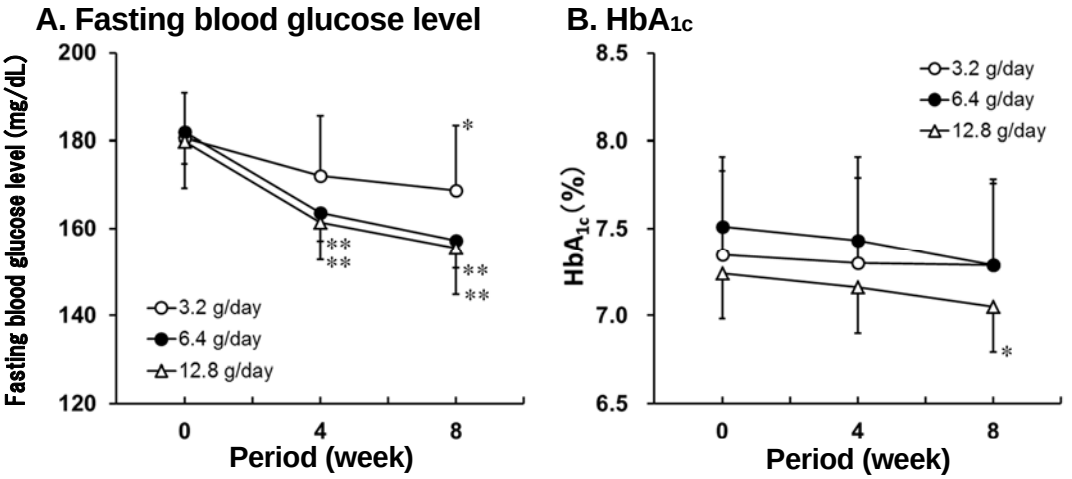
3-3-1. Dose-finding Study (Continuous Intake Study in Patients with Type 2 Diabetes)[11)]

<Continuous Intake Study>

A dose-finding study was conducted in patients with type 2 diabetes (n = 32) using a food product containing Caiapo potato. In this study, the daily intake of Caiapo potato was determined to be 3.2 g/day, 6.4 g/day, and 12.8 g/day.

The parameters such as fasting blood glucose level and HbA_{1c} were measured before and after intake in order to evaluate the efficacy.

The results are shown in **Figures 9** (**A. Fasting blood glucose level**, and **B. HbA_{1c}**).



Mean±SE (n=10-12): * $p < 0.05$, ** $p < 0.01$ *vs*. Before Intake

Figure 9 Effects of Caiapo on Fasting Blood Glucose Level and HbA_{1c}

A significant decrease in the fasting blood glucose level was observed in the 3.2 g/day group at Week 8 ($p < 0.05$). In the Caiapo groups with a daily intake of 6.4 g or greater, a significant decrease was observed at Week 4 and later ($p < 0.01$ for both groups).

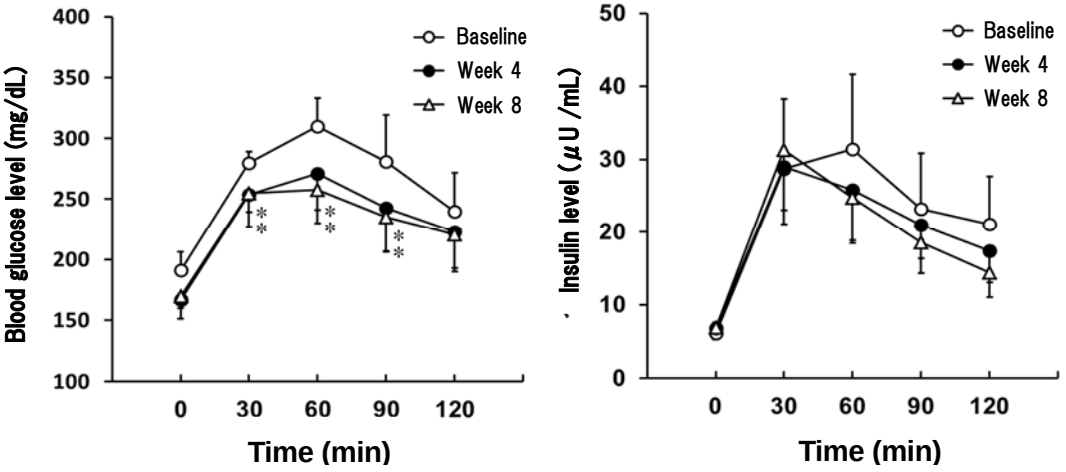
A tendency for the HbA_{1c} level to decrease ($p < 0.1$) was observed in the 6.4 g/day group, and a significant decrease ($p < 0.05$) was observed in the 12.8 g/day group at Week 8.

<Oral Glucose Tolerance Test (OGTT)>

Glucose tolerance tests (glucose load amount: 75 g) were performed in 5 subjects in the Caiapo 6.4 g/day group at baseline, Week 4, and Week 8.

The results of the OGTT are shown in **Figures 10** (**A. blood glucose level**, and **B. insulin level**).

A. Blood glucose level **B. Insulin level**



Mean±SE (n=5): * $p < 0.05$ *vs*. Baseline

Figure 10　OGTT in the Caiapo (6.4 g/day Group)

At Week 4 and later, a significant suppression of hyperglycemia was observed from 30 to 90 minutes after loading ($p < 0.05$ for both). In addition, a significant decrease was shown in the area under the curve for glucose (AUC_G) at Week 8 (66±14 mg·hr/dL) in comparison with that of baseline (80±13 mg·hr/dL) ($p < 0.05$).

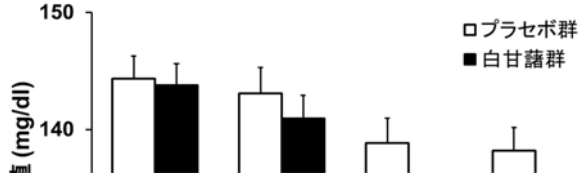
Although no significant difference was observed, the insulin level was decreased at Week 4 and later, suggesting that insulin sensitivity had improved.

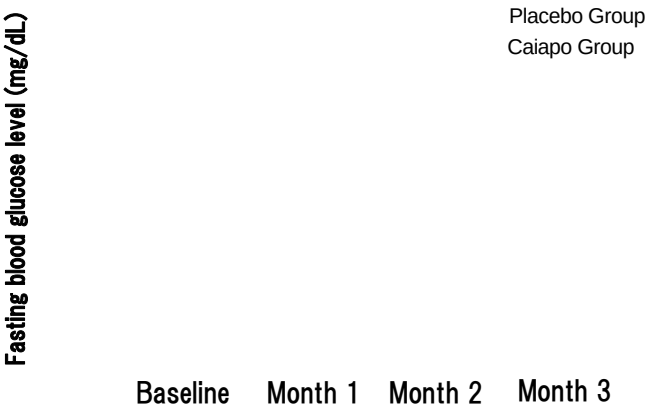
In conclusion, this study demonstrated that Caiapo at a daily intake of 3.2 g or greater was effective for the treatment of hyperglycemia in diabetic patients.

3-3-2. Effectiveness for Obese Patients with Type 2 Diabetes (12-week Intake Study)[12)]

A 12-week intake study using a double-blind method was conducted in 61 obese patients (BMI: ≥ 25 kg/cm^2) with type 2 diabetes. Efficacy was evaluated by comparing the fasting blood glucose levels at baseline, Month 1, Month 2, and Month 3 after intake, blood glucose level at 2 hours after loading in the glucose tolerance test (glucose load amount: 75 g), and HbA_{1c}.

The results for each parameter are shown as follows.

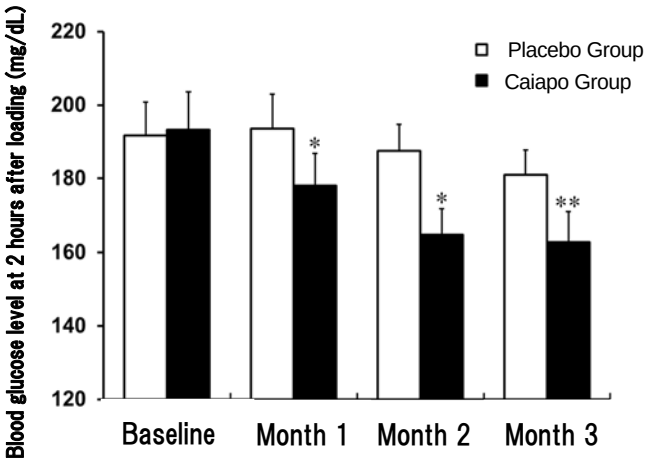




Mean±SE (n=30-31): * $p < 0.05$, ** $p < 0.001$ *vs*. Placebo Group

Figure 11 Effect of Caiapo Intake on Fasting Blood Glucose Level (mg/dL)

In the Caiapo group, fasting blood glucose levels were significantly decreased at Month 2 and Month 3 in comparison with those in the placebo group (Month 2: $p < 0.05$; Month 3: $p < 0.001$).



Mean±SE (n=30-31): * $p < 0.005$, ** $p < 0.001$ *vs*. Placebo Group

Figure 12 Effect of Caiapo Intake on 2-h Glucose Levels (mg/dL)

Blood glucose levels at 2 hours after glucose loading (2-h glucose levels) were significantly decreased at Month 1 in the Caiapo group relative to the placebo group (Month 1: $p < 0.005$; Month 2: $p < 0.005$; Month 3: $p < 0.001$). In the step prior to the onset of diabetes, an increase in postprandial blood glucose levels occurs, in which a decrease in insulin secretion and a decrease in insulin sensitivity in the peripheral tissue are involved.

In the Caiapo group, the fasting blood glucose level was significantly decreased at Month 2 and later (**Figure 11**), and the 2-h glucose level was significantly lowered at Month 1 and later (**Figure 12**). This change suggests that insulin sensitivity and blood glucose control were improved

by the ingestion of Caiapo.

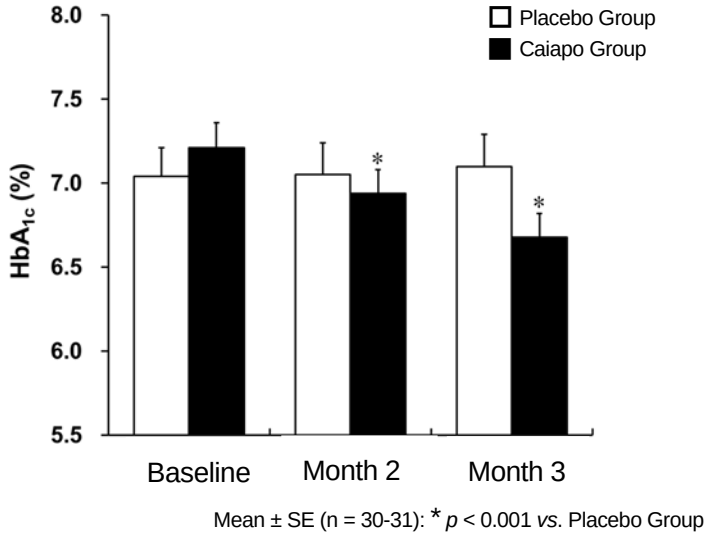


Figure 13 Effect of Caiapo Intake on HbA_{1c} (%)

In the Caiapo group, the HbA_{1c} levels significantly decreased at Month 2 and Month 3 in comparison with those in the placebo group ($p < 0.001$ for both) suggesting that the blood glucose was well controlled during the study period (**Figure 13**).

In conclusion, this study demonstrated that Caiapo ingestion is beneficial for blood glucose control in obese patients with type 2 diabetes.

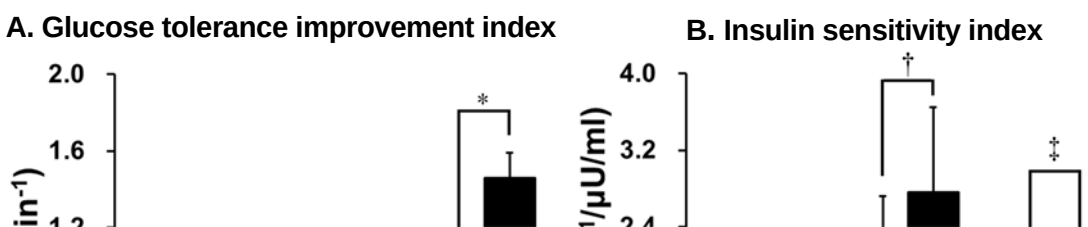
3-3-3. Mechanism of Action (Patients with Type 2 Diabetes)[13)]

<Frequently Sampled Intravenous Glucose Tolerance Test (FSIGT)>

To evaluate the insulin resistance after Caiapo ingestion for 6 weeks (low-dose group: 2 g/day; high-dose group: 4 g/day), a frequently sampled intravenous glucose tolerance test called the minimal model method (hereafter "FSIGT") was performed in patients with type 2 diabetes whose blood glucose level was controlled by dietary therapy. Specifically, glucose (0.3 g/kg) was loaded intravenously during fasting, and, 20 minutes later, additional insulin was loaded (0.05 U/kg). Thereafter, frequent blood sampling was performed to measure insulin levels, and a kinetic analysis was performed to calculate the insulin sensitivity index.

A significant increase (improvement) was observed in the glucose tolerance improvement index (K_G: % min^{-1}) and insulin sensitivity index in the high-dose group. In the low-dose group, a significant increase (improvement) was observed only in the insulin sensitivity index (S_I: 10^4 min^{-1}/μU/mL).

The results are shown in **Figures 14** (**A. K_G** and **B. S_I**).



Mean±SE (n=30-31): * $p < 0.02$, † $p < 0.05$, ‡ $p < 0.021$ *vs*. Before

Figure 14　Effects of Caiapo on Glucose Tolerance and Insulin Sensitivity

Accordingly, these results suggest that the insulin sensitivity was improved by Caiapo ingestion.

In conclusion, FSIGT suggests that Caiapo ingestion contributes to well-controlled blood glucose levels through the improving lipid metabolism and insulin sensitivity in diabetic patients.

3-4. Other Effects

3-4-1. Adiponectin Secretion Promoting Effect[14)]

Retinopathy, nephropathy, and neuropathy are known as the 3 major complications of diabetes, and they are mainly caused by small vessel diseases. In addition, hyperglycemia associated with diabetes also affects large vessels in the form of arteriosclerosis. Although arteriosclerosis can be caused by diseases other than diabetes, such as dyslipidemia and hypertension, it is known that arteriosclerosis tends to develop in diabetic patients and it is considered to be another complication of diabetes.

Heart disease and cerebrovascular disease are the respective No.2 and No.3 causes of death in Japanese[15)]. However, in consideration of the fact that arteriosclerosis is the major cause of these diseases, it can be considered that arteriosclerosis is a life-threatening and very serious condition.

Given this, a study was conducted to investigate the association between adipocytokines (cytokines secreted from adipocytes) and arteriosclerosis, and it was demonstrated that adiponectin, in particular, plays an important role in the development of arteriosclerosis[16)]. This result suggests that adiponectin is effective in the prevention of arteriosclerosis, because it not only increases insulin sensitivity, but also promotes fat-burning and suppresses thrombogenesis.

The effects of Caiapo on the secretion of adiponectin were evaluated in a 5-month intake study using a double-blind method in 61 patients with type 2 diabetes.

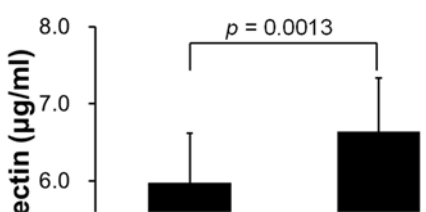


Figure 15 Effect of Caiapo Intake on Adiponectin

The results demonstrated that the secretion of adiponectin was increased in the Caiapo group (**Figure 15**).

In conclusion, this result suggests that Caiapo is an effective food product for the prevention of diabetes and its complications.

3-4-2. Immuno-stimulatory Effect[17)]

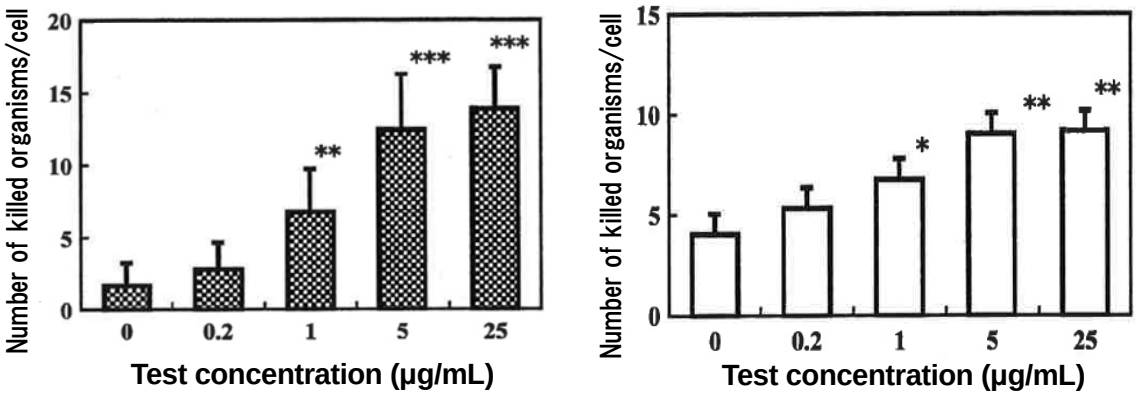
The immune system protects against invasion by foreign organisms such as viruses, bacteria, and molds. Leucocytes, particularly neutrophils and monocytes, play an important role in the immune system. Neutrophils and monocytes provide biological defense by coming into contact with foreign organisms, such as bacteria and fungi, and phagocytizing and sterilizing them.

However, in hyperglycemic conditions (≥ 200 mg/dL), the functions of neutrophils and monocytes (chemotactic activity, phagocytic activity, killing activity, etc.) are compromised[18)]. For this reason, elderly patients and those with chronic diseases such as diabetes prone to infections such as influenza and pneumonia as this protection system (immune system) is compromised.

In this study, killed *Staphylococcus aureus* were coated with the test substance (Caiapo) and cultured with neutrophils (or monocytes) isolated from human peripheral blood to evaluate the immune-stimulatory activity, using the number of killed organisms of *Staphylococcus aureus* phagocytized by neutrophils (or monocytes) as an index of phagocytic activity.

The results are shown in **Figure 16** (**A. Neutrophils**, and **B. Monocytes**).

A. Neutrophils **B. Monocytes**



* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ *vs.* 0 μg/mL

Figure 16 Effects of Caiapo on the Phagocytic Activity of Neutrophils and Monocytes

This study demonstrated that Caiapo could significantly activate the phagocytic activity of neutrophils and monocytes at a level of 1 μg/mL or greater.

In conclusion, these suggest that Caiapo is a food product that can assist diabetic patients in terms of not only blood glucose control, but also immune-stimulation.

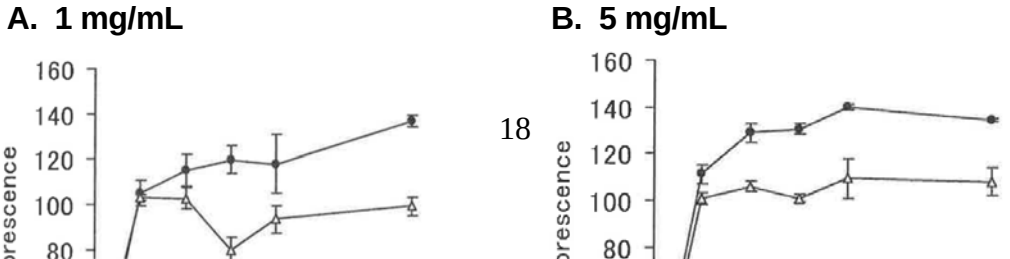
3-4-3. Amyloid Beta Aggregation Inhibiting Effects[19)]

Alzheimer's disease is one of a group of degenerative diseases involving cerebral atrophy that gradually develop from memory impairment to dementia. It has been reported that, in comparison with non-diabetic people, diabetic patients have twice the risk of developing Alzheimer's disease[20)], and this suggests that hyperglycemia and insulin resistance in association with diabetes may induce increased oxidative stress and decreased energy metabolism to promote amyloid beta proteolysis. In fact, there are case reports of improvement in cognitive impairment with treatment of diabetes supporting the evidence of these mechanism[21)].

Amyloid beta protein (Aβ) is a major component of senile plaque amyloid and cerebrovascular amyloid deposited in the brain with Alzheimer's disease. It has been suggested that the aggregation of Aβ into oligomers or fibrils may be a cause of degeneration of synapses and neurons in patients with Alzheimer's disease.

In this study, the inhibitory effect of Caiapo (deproteinized extract powder) against Aβ aggregation was evaluated. The measurement of Aβ fibril and detection of Aβ oligomer were evaluated by the Thioflavin T (ThT) method and Western blot method using Aβ antibodies, respectively.

The results of analysis by the ThT method on the effect of Aβ fibril formation are shown in **Figure 17** (**A. 1 mg/mL**, **B. 5 mg/mL** [test concentration of Caiapo]).



●: Control; ○: Rifampicin (Positive Control, 0.4 mg/mL); ▲: Caiapo; △: Larch AG (Competitor's Product)

Figure 17 Effect of Caiapo on Aβ Fibril Formation

The effects of Caiapo on Aβ fibril formation were weak in comparison with the positive control, but inhibitory effects were observed at both levels of 1 mg/mL and 5 mg/mL.

The effect on Aβ oligomer formation was then evaluated by a Western blotting method using Aβ antibodies. Oligomer formation was evaluated as inhibited if the level of Aβ monomer did not change. The test results are shown in **Figure 18**.

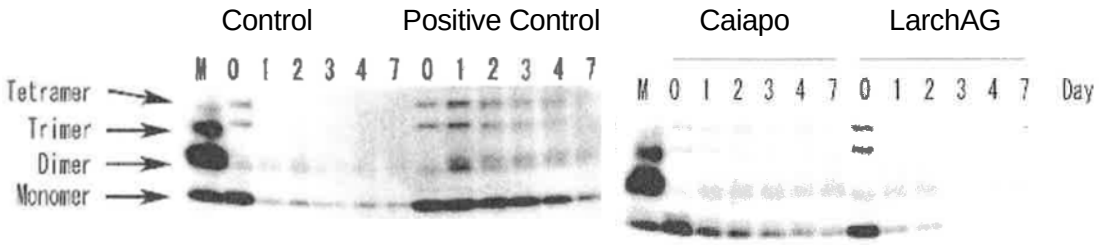


Figure 18 Effect of Caiapo on Aβ Oligomer Formation

In comparison with the positive control, Caiapo rapidly decreased Aβ monomer from Day 1. However, as Aβ monomer was detected up to Day 4, it is demonstrated that Caiapo has a weak inhibitory action on the formation of Aβ oligomer.

In conclusion, these suggest that Caiapo is effective not only for blood glucose control in diabetic patients, but also for the prevention of Alzheimer's disease and dementia.

4. Safety Profile of Caiapo

Caiapo (*Ipomoea batatas* L.) is a variety of *Ipomoea batatas* L. (sweet potato) native to Latin America. In Latin America, it has been mainly used for food, and its tuberous roots and leaves have also been used for the treatment of bleeding and diseases such as hypertension, and diabetes in traditional local medicine[22]. In Japan, Caiapo is cultivated in some areas and has been used as a raw material for sake brewing and healthy foods.

There has been no serious adverse effect reported (as of 2015) in the 20 years since we started marketing Caiapo in April, 1995. In addition, we conducted a questionnaire survey with approximately 2,000 persons, and there have been no reported cases of serious adverse effects such as deconditioning, nor of hypoglycemic symptoms. These support the evidence that Caiapo is a safe food product.

As mentioned above, the safety of Caiapo is backed by a long history of it being eating as foods, however, we decided to further ensure its safety by validating the evidence in non-clinical and clinical studies.

Table 4 shows the safety tests conducted so far and their results.

Table 4. List of Safety Tests and Results

Test	Test Method	Results
Ames test[23]	Test bacteria: *Salmonella typhimurium* TA1535, TA1537, TA98, TA100, and *Escherichia coli* WP2uvrA$^-$ Test substance: Caiapo Test concentrations: 312.5, 625, 1250, 2500, and 5000 μg/plate	Negative
Chromosomal Aberration Test [24]	Cells: Fibroblasts of the lung of Chinese hamster (CHL/IU) Test substance: Caiapo Test concentrations: 0, 625, 1250, 2500, and 5000 μg/mL	Negative
Acute Toxicity Test [25]	Animal: SD rats (male and female, 5 in each group) Test substance: Caiapo	NOAEL Male/Female: ≥ 2 g/kg
Subchronic test (13-week) [26]	Animal: SD rats (male and female, 10 in each group) Test substance: Caiapo Dosages: 0.2%, 1.0%, and 5.0% (in the diet)	NOAEL Male : ≥ 3.4 g/kg Female: ≥ 3.8 g/kg
Long-term intake Test (12-week) [27]	Subject: Patients with borderline diabetes (n=66) Intake amount: Caiapo 6.24 g/day	No abnormal finding
Long-term intake Test (13-week) [28]	Subject: Patients with borderline/mild type 2 diabetes (n=31) Intake amount: Caiapo 6.24 g/day	No abnormal finding
Long-term intake Test (6-month) [29]	Subject: Healthy volunteers and patients with type 2 diabetes (n=23) Intake amount: Caiapo 6.24 g/day	No abnormal finding
Overdose test (30 days) [30]	Subject: Healthy volunteers (n=19) Intake amount: Caiapo 18.72 g/day (3 times the recommended amount)	No abnormal finding

The non-clinical and clinical studies for safety evaluation found no abnormal data related to

Caiapo. These findings showed that continuous administration of Caiapo does not cause any clinical problem.

In conclusion, these studies suggest that Caiapo is a food product with an established safety profile.

5. Stability

A 30-month long-term study was conducted to evaluate the stability of Caiapo.

Specifically, Caiapo was sealed in aluminum packages and stored at room temperature (19-26°C) for 30 months for the following 8 test items: description, identification (coloration reactions), purity (heavy metals, arsenic), loss on drying, total ash, active ingredient content, total viable count, and *Escherichia coli* count.

These tests found no significant changes in any of the test items at Month 30,

In conclusion, this study suggests that Caiapo has a quality preservation period of 24 months, calculated on the basis of safety factor 0.8.

6. Conclusion

To validate the effects of Caiapo, tests were performed in spontaneously diabetic animal models (Zucker *fa/fa* rats, *KKA*^{y} and *db/db* mice) and diabetic patients. These tests demonstrated that Caiapo improves insulin resistance, giving rise to a hypoglycemic action.

Non-clinical and clinical studies showed the absence of any adverse effects of Caiapo.

Caiapo is an innovative food product with superior antidiabetic effect and it can be used at ease.

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