Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : November 7, 2017
Accepted : December 6, 2017
Published online : December 31, 2017

Original article

Safety evaluation of excess or long-term intake of food containing a Salacia extract.

Nobuko Kajiwara [1)], Ken-ichi Onodera [1)], Tomoko Tsuji [1)], Yoshikazu Yonei [2)]

1) Yoshinoya Holdings Ltd, Tokyo, Japan
2) Anti-Aging Medical Research Center and Glycative Stress Research Center, Graduate School of Life and Medical Sciences, Doshisha University, Kyoto, Japan

Abstract

***Objectives*:** To evaluate the safety of gyudon topping (cooked beef and onion in a soy sauce-based tare sauce, often eaten with a bowl of steamed rice), containing a Salacia extract, an excess intake trial (three times the regular intake) and a long-term intake trial (over 12 weeks) were performed.

***Methods*:** This study included 32 healthy subjects (17 men and 15 women; mean age, 39.8 ± 12.5 years) in the excess intake trial and 32 healthy subjects (17 men and 15 women; mean age, 41.9 ± 10.6 years) in the long-term intake trial. All subjects were within the age range of 20 to 64 years. The fasting blood glucose levels of all subjects were below 125 mg/dL. The sodium and fat contents of the test food were reduced by 25% compared with the existing product.

***Results*:** No adverse effects were observed in either trial. Clinically significant changes were not detected in physical examinations or blood tests.

***Conclusion*:** The safety of gyudon topping with a Salacia extract in trials of excess and long-term intake was confirmed. The gyudon topping described in this study is safe and non-detrimental for healthy individuals and those who are mindful of postprandial blood glucose elevation.

***KEY WORDS*:** safety, clinical study, Salacia, salacinol

Introduction

Diabetes is predominantly classified into type 1 and type 2 by onset factors. Approximately 95% of people with diabetes in Japan are believed to have type 2 diabetes. Postprandial hyperglycemia is an important indicator in type 2 diabetes, but also in prediabetes.

According to the Japanese National Health and Nutrition Survey in 2016, the number of people who are strongly suspected to have diabetes and the number of patients with diabetes have each reached 10 million. Epidemiological studies have shown that postprandial hyperglycemia is a direct and independent risk factor for cardiovascular disease, because the elevated level of blood glucose after a meal, which is called a "glucose spike," injures the vascular endothelium.

The prevention of postprandial hyperglycemia reduces the risk of both diabetes and cardiovascular disease. This led to the development of a new functional food for the suppression of postprandial hyperglycemia, a gyudon topping containing the extract of *Salacia reticulata* (of the family Hippocrateaceae). A single intake of this new functional gyudon topping with steamed rice resulted in the suppression of postprandial hyperglycemia compared with that after the intake of steamed rice and the topping without the Salacia extract [1,2].

S. reticulata grows in subtropical regions, such as India and Sri Lanka, and has been used for the treatment of the early stage of diabetes in Ayurvedic medicine, the Indian traditional medicine system, for 3000 years. Pharmacological studies have demonstrated that Salacia roots modulate multiple targets, including peroxisome proliferator-activated receptor α (PPAR-α)-mediated lipogenic gene transcription, angiotensin II/angiotensin II type I receptor, α-glucosidase, aldose reductase, and pancreatic lipase [3]. Yoshikawa *et al.* [4,5] reported that one of the active components

Contact Address: Nobuko Kajiwara
Yoshinoya Holdings Ltd, Merchandising Division
Daiwa Rivergate 18F, 36-2 Nihonbashi Hakozaki-cho, Chuo-ku, Tokyo 103-0015, Japan
Phone: +81-3-5651-8779 Fax: +81-3-5651-8778 Mail: n.kajiwara@ysn.yoshinoya.com
Co-authors: Onodera K, k.onodera@ysn.yoshinoya.com ; Tsuji T, t.tsuji@ysn.yoshinoya.com ; Yonei Y, yyonei@mail.doshisha.ac.jp

responsible for α-glucosidase inhibition was salacinol, a sulfonium constituents.

To determine the safety of the Salacia extract, Ames tests, acute toxicity tests, antigenicity and phototoxicity tests [6], and a repeated dose toxicity test at a maximum dose of 1000 mg/kg in rats [7] were conducted. In addition, several clinical studies on the efficacy and safety of Salacia have been conducted using tablets, granules, and beverages. However, the safety of the Salacia extract added into gyudon toppings was unknown.

In the present study, trials on the intake of gyudon topping with excessive (three times the regular dose) Salacia extract for 4 weeks (**Trial A**) and with a regular dose of Salacia extract for 12 weeks (**Trial B**) were performed to elucidate the safety and effects on parameters related to the metabolic syndrome.

Materials and Methods

This study consisted of two parts: **Trial A**, an excess intake trial (three times the regular amount); and **Trial B**, a long-term intake trial.

Subjects

A total of 32 healthy subjects (17 men and 15 women; mean age, 39.8 ± 12.5 years) were enrolled in **Trial A**, and a total of 32 healthy subjects (17 men and 15 women; mean age, 41.9 ± 10.6 years) were enrolled in **Trial B**. All subjects were aged between 20 and 64 years, with fasting blood glucose levels below 125 mg/dL.

In both trials, the following exclusion criteria were applied: subjects with alcohol intake of more than 20 g/day; subjects currently under treatment at a hospital or receiving medication for the purposes of treatment; subjects with dietary restrictions; subjects with a serious hepatic, kidney, or cardiac disorder; subjects with cerebrovascular disease; subjects with organ damage; subjects who were allergic to the test food; subjects who were pregnant, possibly pregnant, or breastfeeding; and subjects judged inappropriate by the supervising doctor.

The subjects were fully informed of the contents and methods of the study, and written informed consent was obtained from all participants.

The design of **Trials A** and **B** was based upon the Declaration of Helsinki and approved by Ageo Kousei Hospital Ethics Review Committee (**Trial A**: UMIN Registration No. UMIN000026089; **Trial B**: UMIN Registration No. UMIN000026083).

Foods for the studies

The composition of the test foods is shown in ***Table 1***. The hot-water extract from the stems of S. reticulata was used. The test food comprised frozen gyudon toppings, which were prepared by using the following method: the Salacia extract was dissolved in 1.7% potassium chloride solution, mixed with tare sauce, to which onions and beef were added, and the mixture was heated. The dose of Salacia extract in **Trial A** was three times higher than that used in **Trial B**. The dose of salacinol in **Trial B** was 0.6 mg, which was defined as a regular dose in this study. Caramel pigment was used adjust the color of the foods.

Study design

The studies were randomized, double-blind, placebo-controlled trials. All subjects were randomly assigned to one of two groups in each trial. The subjects consumed gyudon toppings once per day after the products were cooked. During the trial period, the subjects were requested to record the following information in a diary: intake of test food, lifestyle habits (meals, snacks, exercise, drinking, and smoking), and physical condition.

Trial A

Trial A required four visits to the clinic: one for health screening, a pre-intake visit (week 0), and after 2 and 4 weeks of intake. At each visit, the following measurements were obtained: body weight and height to calculate body mass index (BMI), waist circumference, body fat by bio-electrical impedance (BI) analysis; blood pressure; and pulse rate. Additionally, hematology, blood biochemistry, urinalysis, and a medical history review were conducted at each visit. During the trial period, the subjects' daily diets were monitored. The subjects recorded every meal in detail for the 3 days immediately prior to the clinic visits in week 0 and week 4.

Trial B

Trial B required five visits to the clinic: one for health screening, a pre-intake visit (week 0), and after 4, 8, and 12 weeks of intake. The same measurements were obtained as

Table 1. Composition of the test foods for the excess and the long-term intake trial.

Item	Unit	Content in 1 package		
		Salacia for the excess intake trial	Salacia for the long-term intake trial	Placebo for both trials
Energy	kcal	278	275	273
Protein	g	13.0	12.3	13.9
Fat	g	22.1	22.0	20.9
Carbohydrate	g	6.9	7.0	7.0
Sodium	mg	713	662	691
Salacinol	mg	1.8	0.6	0

for Trial A; in addition, pentosidine was measured at weeks 0 and 12 Pentosidine was measured using a commercially available kit (Fushimi Pharmaceutical Co., Ltd.). Daily diet surveys were conducted at the visits in weeks 0, 4, 8, and 12.

Statistical analyses

The data are presented as the mean ± standard deviation. Two-way analysis of variances (ANOVA) was used to examine the statistical significance. Significant differences in two-way ANOVA were further analyzed by in-group and between-group comparisons. Comparisons between the Placebo and Salacia groups were conducted by Student's t-test. Comparisons between before intake and after 4, 8, and 12 weeks of intake in each group were conducted by the Bonferroni test. The Wilcoxon signed-rank test was used to compare urinalysis parameters at each measurement point with those at week 0. The Mann-Whitney U-test was used for the comparison of the Placebo and Salacia groups.

All statistical analyses were computed by using SPSS Statistics 22 (IBM Japan Ltd, Minato-ku and Tokyo, Japan). A significance level of less than 5% in two-sided testing was considered.

Results

Subjects

Trial A: Excess Intake Trial (1.8 mg/day for 4 weeks)

In total, 16 subjects (8 men and 8 women) from the placebo group and 16 subjects (9 men and 7 women) from the Salacia group started **Trial A**. One subject from the Placebo group dropped-out during the trial period owing to personal circumstances. All data, including those acquired from the dropped-out subject, were analyzed. The mean compliance rate for test food consumption by the subjects was 100% ± 0.0% in the Placebo group and 99.8% ± 1.0% in the Salacia group.

Trial B: Long-term intake trial (0.6 mg/day for 12 weeks)

In total, 16 subjects (8 men and 8 women) from the Placebo group and 16 subjects (9 men and 7 women) from the Salacia group started **Trial B**. One subject from the Placebo group and one subject from the Salacia group dropped-out during the trial period. The reasons for the drop-out were compliance matter violation and personal circumstances. As it was judged that these had no causal relationship with the study, all data, including those from the dropped-out subjects, were analyzed. The mean compliance rate for test food consumption by the subjects was 99.9% ± 0.6% in the Placebo group and 100% ± 0.1% in the Salacia group.

Diet survey

The mean daily nutritional compositions consumed by the subjects in **Trial A** and **Trial B** are shown in ***Table 2*** and ***3***, respectively.

Trial A

A significant difference in cholesterol level was observed at week 0 between the two groups. It was assumed that the difference was a result of the dietary habits before starting the trial in the Placebo group. No difference was observed after 4 weeks.

At week 0, the percentages of protein, fat, and carbohydrate to total energy were 14.6 ± 1.7%, 31.6 ± 4.1%, and 51.0 ± 4.2% in the Salacia group and 14.8 ± 1.4%, 33.2 ± 4.1%, and 49.7 ± 5.4% in the Placebo group, respectively. After 4 weeks, the percentages were 14.1 ± 1.8%, 33.9 ± 4.7%, and 50.2 ± 5.8% in the Salacia group and 14.5 ± 2.0%, 34.0 ± 4.6%, and 49.6 ± 5.6 % in the Placebo group, respectively. No significant differences were observed in the total energy and the ratio of PFC balance between both groups.

Table 2. Nutritional intake during the excess intake trial (Trial A).

Item	Unit	Group	week 0	week 4
Energy	kcal	Salacia	1850 ± 399	1906 ± 339
		Placebo	2085 ± 458	2025 ± 351
Protein	g	Salacia	67.6 ± 17.2	67.7 ± 17.5
		Placebo	76.8 ± 17.6	73.7 ± 19.4
Fat	g	Salacia	65.3 ± 18.2	72.1 ± 18.3
		Placebo	76.9 ± 19.3	76.8 ± 19.3
Carbohydrate	g	Salacia	234.7 ± 50.3	237.6 ± 40.3
		Placebo	259.6 ± 64.2	249.4 ± 42.1
Cholesterol	mg	Salacia	301.0 ± 112.5	296.3 ± 151.3
		Placebo	405.2 ± 134.4 *	340.2 ± 140.3
Fiber	g	Salacia	10.2 ± 4.1	10.6 ± 3.1
		Placebo	12.2 ± 3.8	11.9 ± 4.5
Salt	g	Salacia	8.5 ± 1.9	8.1 ± 2.0
		Placebo	8.9 ± 1.6	9.0 ± 2.0

Data are expressed as the mean ± SD. * $p < 0.05$ in comparison with the Placebo by Student's t-test. Salacia group, n = 16 (9 males and 7 females); Placebo group, n = 16 (8 males and 8 females). SD, standard deviation.

Table 3. Nutritional intake during the long-term intake trial (Trial B).

Item	Unit	Group	week 0	week 4	week 8	week 12
Energy	kcal	Salacia	1775 ± 369	2036 ± 565	2038 ± 576	2088 ± 674
		Placebo	1823 ± 358	1916 ± 270	1969 ± 262	2000 ± 412
Protein	g	Salacia	65.8 ± 13.1	72.8 ± 17.5	71.6 ± 16.1	72.1 ± 17.2
		Placebo	64.4 ± 14.7	71.8 ± 12.9	71.1 ± 10.8	72.4 ± 15.0
Fat	g	Salacia	60.8 ± 15.9	72.8 ± 16.8	70.1 ± 17.9	69.9 ± 16.2
		Placebo	64.9 ± 20.1	71.6 ± 16.0	70.7 ± 12.8	73.6 ± 20.1
Carbohydrate	g	Salacia	225.0 ± 51.6	258.9 ± 115.1	264.5 ± 115.8	278.9 ± 138.0
		Placebo	233.7 ± 61.0	234.1 ± 43.9	250.3 ± 52.2	249.8 ± 67.4
Cholesterol	mg	Salacia	309.3 ± 124.7	364.2 ± 181.0	313.7 ± 188.8	295.2 ± 161.9
		Placebo	351.1 ± 172.2	333.4 ± 121.2	335.1 ± 161.1	323.0 ± 167.3
Fiber	g	Salacia	10.4 ± 2.8	10.8 ± 4.1	10.0 ± 3.1	9.1 ± 2.7
		Placebo	10.6 ± 3.6	9.8 ± 3.9	9.6 ± 2.3	9.7 ± 3.6
Salt	g	Salacia	9.0 ± 1.9	9.8 ± 2.8	8.8 ± 1.6	9.6 ± 2.3
		Placebo	8.4 ± 1.5	8.3 ± 1.5	9.1 ± 1.9	8.6 ± 2.2

Data are expressed as the mean ± SD. No significant difference. Salacia group, n = 16 (9 males and 7 females); Placebo group, n = 16 (8 males and 8 females). SD, standard deviation.

Trial B

No significant differences between the Placebo and Salacia groups were observed.

At 0 week, the percentages of protein, fat, and carbohydrate to total energy were 14.9 ± 1.4%, 30.7 ± 4.7%, and 50.7 ± 5.4% in the Salacia group and 14.3 ± 3.1%, 32.0 ± 7.2%, and 51.0 ± 8.9% in the Placebo group, respectively. After 12 weeks of consumption, the percentages were 14.2 ± 2.2%, 31.1 ± 6.0%, and 52.0 ± 8.1% in the Salacia group and 14.6 ± 2.4%, 33.3 ± 6.6%, and 49.6 ± 7.6% in the Placebo group, respectively.

Safety evaluation

Adverse events

The adverse events observed during the trial period are shown in ***Table 4*** and ***5***. No serious adverse events were observed.

Trial A: Two adverse events were reported.

In the Salacia group, one case of mild trophic liver dysfunction occurred in a female subject. Slightly abnormal values of 49 U/L, 49 U/L, and 33 U/L were observed for AST, ALT, and γ-GT, respectively, on day 12. The doctor judged that there was a possibility of the excess intake of foods other than the test food after investigation of the subject's situation. The subject continued the trial, and all values returned to normal at day 27. These events were considered to be unrelated to the test food consumption.

Another event in the Salacia group was the occurrence of herpes zoster in a female subject. She took medicines and recovered without interruption in test food intake. As this was a viral disease, it did not prevent the patient from consuming the test food. No adverse events occurred in the Placebo group.

Trial B: Five adverse events were reported.

In the Salacia group, symptoms of cold were reported by a female subject, and allergic rhinitis and menstrual pain (twice) were reported by another female subject. These events were considered to be unrelated to the test food consumption. In the Placebo group, two cases (in females) with symptoms of cold were reported. These events were also considered to be unrelated to the test food consumption.

Physical parameters

The measurements of physical parameters in the two trials are shown in ***Tables 6*** and ***7***

Trial A

In the Salacia group, body fat significantly decreased with an increase in intake time. The mean body fat percentages at week 0 and 4 were 24.2 ± 8.3% and 22.7 ± 8.7%, respectively ($p < 0.05$). In contrast, no significant difference was observed during the trial period in the Placebo group.

A significant difference was recorded in diastolic blood pressure at week 0 (72.1 ± 7.7 mmHg) and week 4 (66.7 ± 8.3 mmHg) in the Salacia group. In contrast, no significant changes occurred in diastolic blood pressure of the Placebo group. In both groups, no other significant differences were observed.

Trial B

In the Salacia group, the mean body fat was lower at week 12 than at week 0, but the difference was not

Table 4. Adverse events and subjective symptoms during the excess intake trial (Trial A).

Group	Number	Sex	Apperance day and disappearance day from start of intake	Event	Treatment	Degree	Outcome	Seriousness
Salacia	2	Female	Day 12 to Day 27	Mild trophic liver dysfunction	None	Mild	Recovered	Not serious
		Female	Day 21 to Day 28	Herpes zoster	Doctor consultation and medication	Moderate	Recovered	Not serious

Table 5. Adverse events and subjective symptoms during the long-term intake trial (Trial B).

Group	Number	Sex	Apperance day and disappearance day from start of intake	Event	Treatment	Degree	Outcome	Seriousness
Salacia	3	Female	Day 5 to Day 7	Cold symptoms	None	Mild	Recovered	Not serious
		Female	Day 3 to Day 6	Menstrual pain	Medication	Mild	Recovered	Not serious
			Day 9 to Day 37	Allergic rhinitis	Medication	Mild	Recovered	Not serious
			Day 68 to Day 71	Menstrual pain	Medication	Mild	Recovered	Not serious
Placebo	2	Female	Day 28 to Day 30	Cold symptoms	None	Mild	Recovered	Not serious
		Female	Day 29 to Day 31	Cold symptoms	Medication	Mild	Recovered	Not serious

Table 6. Measurements of physical parameters during the excess intake trial (Trial A).

Item	Unit	Group	week 0	week 2	week 4	
Weight	kg	Salacia	63.3 ± 9.4	63.1 ± 9.6	63.0 ± 9.6	
		Placebo	60.7 ± 11.3	60.6 ± 11.3	60.8 ± 11.8	
BMI	kg/m^2	Salacia	22.5 ± 2.8	22.5 ± 2.8	22.4 ± 2.9	
		Placebo	22.1 ± 2.7	22.0 ± 2.7	22.2 ± 2.8	
Body fat	%	Salacia	24.2 ± 8.3	24.1 ± 8.0	22.7 ± 8.7	#
		Placebo	23.7 ± 7.8	23.7 ± 7.7	24.2 ± 8.3	
Waist circumference	cm	Salacia	81.0 ± 8.7	81.8 ± 8.3	81.4 ± 9.3	
		Placebo	79.7 ± 7.3	79.5 ± 7.4	79.6 ± 7.8	
Body temperature	°C	Salacia	36.3 ± 0.3	36.1 ± 0.5	36.2 ± 0.4	
		Placebo	36.1 ± 0.6	36.1 ± 0.6	36.2 ± 0.5	
Systolic blood pressure	mmHg	Salacia	114.5 ± 10.1	111.9 ± 7.9	111.3 ± 12.3	
		Placebo	110.5 ± 8.2	111.3 ± 10.5	110.5 ± 12.4	
Diastolic blood pressure	mmHg	Salacia	72.1 ± 7.7	71.0 ± 8.0	66.7 ± 8.3	##
		Placebo	70.3 ± 8.3	69.2 ± 11.3	65.8 ± 10.8	
Pulse rate	bpm	Salacia	67.4 ± 9.3	68.1 ± 9.1	70.8 ± 10.1	
		Placebo	73.0 ± 8.6	72.4 ± 11.3	74.6 ± 11.4	

Data are expressed as the mean ± SD. # $p < 0.05$, ## $p < 0.01$ in comparison with week 0 by the Bonferroni test. Salacia group, n = 16 (9 males and 7 females); Placebo group, n = 16 (8 males and 8 females). SD, standard deviation.

Table 7. Measurements of physical parameters during the long-term intake trial (Trial B).

Item	Unit	Group	week 0	week 4	week 8	week 12
Weight	kg	Salacia	65.5 ± 15.9	67.0 ± 15.5	66.6 ± 15.6	66.9 ± 16.1
		Placebo	65.6 ± 13.3	66.8 ± 13.0	66.7 ± 13.3	66.4 ± 13.1
BMI	kg/m^2	Salacia	23.9 ± 4.8	24.3 ± 4.8	24.2 ± 4.8	24.3 ± 5.0
		Placebo	23.7 ± 3.4	24.1 ± 3.2	24.0 ± 3.3	23.9 ± 3.2
Body fat	%	Salacia	26.1 ± 7.2	26.8 ± 7.6	26.3 ± 7.5	25.8 ± 7.4
		Placebo	25.0 ± 5.8	25.1 ± 6.2	25.4 ± 5.6	24.9 ± 5.9
Waist circumference	cm	Salacia	84.5 ± 11.9	86.0 ± 12.3	85.3 ± 11.7	85.8 ± 12.7
		Placebo	83.3 ± 8.7	84.0 ± 8.3	84.2 ± 8.3	84.6 ± 8.1
Body temperature	°C	Salacia	36.1 ± 0.5	36.1 ± 0.4	36.1 ± 0.4	36.2 ± 0.4
		Placebo	36.5 ± 0.2 *	36.2 ± 0.4	36.4 ± 0.4 *	36.5 ± 0.3 *
Systolic blood pressure	mmHg	Salacia	116.1 ± 14.4	121.0 ± 15.0	119.2 ± 12.0	119.2 ± 14.0
		Placebo	114.6 ± 15.0	118.1 ± 13.3	116.8 ± 10.5	114.0 ± 12.3
Diastolic blood pressure	mmHg	Salacia	75.8 ± 14.0	79.9 ± 14.5	74.2 ± 10.5	73.5 ± 11.0
		Placebo	73.0 ± 12.2	74.3 ± 9.0	70.3 ± 9.3	69.3 ± 10.8
Pulse rate	bpm	Salacia	70.8 ± 13.5	69.1 ± 10.1	65.2 ± 7.3	67.3 ± 9.3
		Placebo	71.3 ± 9.3	66.8 ± 5.7	67.2 ± 6.4	71.3 ± 6.9

Data are expressed as the mean ± SD. * $p < 0.05$ in comparison with the Placebo by Student's t-test. Salacia group, n = 16 (9 males and 7 females); Placebo group, n = 16 (8 males and 8 females). BMI, body mass index; SD, standard deviation.

statistically significant.

The mean diastolic blood pressure was lower at week 12 than at week 0 in both groups, but the difference was not statistically significant.

The mean body temperature in the Placebo group was consistently higher than that in the Salacia group during the trial period, but this was considered to be unrelated the test food consumption.

Hematology and blood biochemistry

The measurements in hematology and blood biochemistry parameters in the two trials are shown in ***Tables 8*** and ***9***.

Trial A

Although the WBC of women in the Placebo group was significantly higher than that in the Salacia group at week 0, it decreased to the same level as in the Salacia group at weeks 2 and 4. Furthermore, the values in all examinations were within the normal range. This indicated that no significant hematological effects were induced by the test food.

The values of LDL-cholesterol, Cl, and HbA1c in the Salacia group randomly fluctuated within the normal range during the trial period, and the fluctuations showed no time dependency. This indicated that the gyudon topping with Salacia exerted no significant effects on these parameters.

At 4 weeks, blood glucose levels decreased in both the Placebo and Salacia groups in comparison with the values at week 0; the change was statistically significant in the Salacia group.

Trial B

During the 12-week trial period, the values of several test parameters varied within the physiologically normal range. Some of these variations were significantly different between the Placebo and Salacia groups at only the middle examination points. These variations were not considered to be directly related to test food consumption. None of the parameters was significantly different between the Placebo and Salacia groups at the final examination.

In the Salacia group, MCV and MCH values in men were significantly decreased, but by a very small degree, at week 12 compared to values at week 0. However, this result was not observed in women.

In contrast, pentosidine in the Placebo group was increased significantly at week 12, but not in the Salacia group. Cl in both groups increased by approximately 2% at week 12. This was presumed to result from the loss of protein associated with blood sampling and compensatory blood concentration. The change was not significant, but supported the similar reduction observed for albumin. These results indicated that the gyudon topping with Salacia exerted no significant effects on these parameters.

Urinalysis

The results of the urine tests in both trials are shown in ***Tables 10*** and ***11***.

Table 8. Measurements of hematological and blood biochemical parameters during the excess intake trial (Trial A).

Item	Unit	Standard value	Group	week 0	week 2	week 4
TG	mg/dL	50-149	Salacia	83.8 ± 53.7	68.9 ± 32.5	94.1 ± 75.9
			Placebo	58.4 ± 11.9	65.0 ± 22.2	62.0 ± 26.2
TC	mg/dL	150-219	Salacia	185.7 ± 27.4	191.2 ± 26.8	185.2 ± 27.0
			Placebo	186.6 ± 27.5	193.1 ± 31.2	187.6 ± 31.4
LDL-C	mg/dL	70-139	Salacia	101.8 ± 20.0	110.1 ± 20.8 #	102.0 ± 20.0
			Placebo	102.6 ± 25.7	108.6 ± 28.8	104.7 ± 30.3
HDL-C	mg/dL	M：40-86	Salacia	53.9 ± 11.6	53.2 ± 9.8	53.0 ± 12.7
			Placebo	59.3 ± 13.6	59.9 ± 14.3	56.9 ± 11.4
		F：40-96	Salacia	75.1 ± 24.4	76.0 ± 21.4	75.0 ± 24.9
			Placebo	80.6 ± 14.4	81.1 ± 13.0	82.1 ± 13.4
TP	g/dL	6.7-8.3	Salacia	7.2 ± 0.3	7.2 ± 0.3	7.2 ± 0.4
			Placebo	7.3 ± 0.2	7.4 ± 0.2	7.4 ± 0.3
ALB	g/dL	3.8-5.2	Salacia	4.5 ± 0.3	4.6 ± 0.3	4.5 ± 0.2
			Placebo	4.4 ± 0.3	4.5 ± 0.3	4.5 ± 0.3
UA	mg/dL	M：3.7-7.0	Salacia	6.4 ± 1.0	6.4 ± 1.1	6.5 ± 1.4
			Placebo	5.8 ± 1.0	5.5 ± 0.9	5.6 ± 0.7
		F：2.5-7.0	Salacia	4.9 ± 0.8	4.7 ± 0.6	5.0 ± 0.6
			Placebo	4.7 ± 1.4	4.7 ± 1.5	4.7 ± 1.4
Cre	mg/dL	M：0.61-1.04	Salacia	0.852 ± 0.143	0.842 ± 0.119	0.858 ± 0.203
			Placebo	0.840 ± 0.097	0.864 ± 0.116	0.840 ± 0.098
		F：0.47-0.79	Salacia	0.596 ± 0.089	0.587 ± 0.081	0.600 ± 0.066
			Placebo	0.578 ± 0.045	0.601 ± 0.047	0.583 ± 0.034
BUN	mg/dL	8.0-22.0	Salacia	12.7 ± 3.4	13.7 ± 4.4	13.2 ± 2.6
			Placebo	13.1 ± 2.8	13.1 ± 2.8	13.4 ± 2.9
AST (GOT)	U/L	10-40	Salacia	19.6 ± 4.3	21.6 ± 8.9	20.8 ± 6.1
			Placebo	19.1 ± 4.8	18.4 ± 4.4	18.7 ± 4.2
ALT (GPT)	U/L	5-40	Salacia	17.3 ± 7.1	21.2 ± 11.8	20.1 ± 10.9
			Placebo	17.7 ± 10.0	16.5 ± 7.2	16.6 ± 6.7
γ-GTP	U/L	M：≦70	Salacia	22.9 ± 8.5	22.9 ± 8.1	24.6 ± 12.6
			Placebo	25.1 ± 10.6	27.3 ± 12.5	25.6 ± 11.3
		F：≦30	Salacia	15.9 ± 4.9	18.9 ± 8.1	16.4 ± 5.9
			Placebo	15.1 ± 7.4	14.9 ± 6.9	15.4 ± 7.7
Glucose	mg/dL	70-109	Salacia	97.9 ± 10.1	96.6 ± 12.7	89.2 ± 6.9 #
			Placebo	95.8 ± 11.1	99.7 ± 15.5	91.1 ± 10.0
LDH	U/L	115-245	Salacia	158.5 ± 22.5	157.7 ± 18.2	161.8 ± 27.0
			Placebo	162.6 ± 29.0	161.2 ± 21.4	165.3 ± 17.1
HbA1c [NGSP]	%	4.6-6.2	Salacia	5.3 ± 0.3	5.2 ± 0.3	5.3 ± 0.2
			Placebo	5.2 ± 0.3	5.2 ± 0.4	5.2 ± 0.3
Insulin	μIU/mL	1.84-12.2	Salacia	8.5 ± 4.5	7.6 ± 4.4	5.4 ± 2.6
			Placebo	10.0 ± 6.1	11.2 ± 8.5	5.7 ± 2.4
Na	mEq/L	136-147	Salacia	140.1 ± 1.7	140.4 ± 2.4	140.3 ± 1.5
			Placebo	140.6 ± 1.5	140.4 ± 1.8	140.3 ± 1.8
Cl	mEq/L	98-109	Salacia	104.3 ± 1.9	105.1 ± 2.4	105.2 ± 2.2 ##
			Placebo	104.5 ± 2.1	104.1 ± 1.0	105.0 ± 1.6
K	mEq/L	3.6-5.0	Salacia	4.1 ± 0.2	4.2 ± 0.2	4.2 ± 0.3
			Placebo	4.2 ± 0.3	4.2 ± 0.4	4.2 ± 0.2

Item	Unit	Standard value	Group	week 0	week 2	week 4
Ca	mg/dL	8.5-10.2	Salacia	9.3 ± 0.3	9.3 ± 0.3	9.4 ± 0.4
			Placebo	9.3 ± 0.3	9.4 ± 0.3	9.3 ± 0.4
Mg	mg/dL	1.8-2.6	Salacia	2.3 ± 0.2	2.3 ± 0.1	2.3 ± 0.1
			Placebo	2.2 ± 0.2	2.2 ± 0.1	2.2 ± 0.1
Fe	μg/dL	M：54-200	Salacia	108.0 ± 45.6	99.8 ± 46.9	85.3 ± 18.9
			Placebo	107.5 ± 54.6	105.0 ± 44.4	101.4 ± 33.2
		F：48-154	Salacia	89.0 ± 36.0	89.6 ± 33.5	80.6 ± 29.2
			Placebo	97.9 ± 61.8	119.9 ± 49.7	120.9 ± 79.3
WBC	/μL	M：3900-9800	Salacia	4844 ± 1747	5500 ± 1179	5756 ± 1526
			Placebo	5300 ± 1294	5500 ± 1338	5814 ± 677
		F：3500-9100	Salacia	5143 ± 902	5357 ± 922	5886 ± 1173
			Placebo	6500 ± 1306 *	5663 ± 703	5663 ± 1249
RBC	$\times 10^4/\mu L$	M：427-570	Salacia	485.8 ± 39.1	485.7 ± 47.0	486.9 ± 36.1
			Placebo	492.1 ± 29.5	497.4 ± 23.3	497.3 ± 32.5
		F：376-500	Salacia	432.4 ± 29.9	427.0 ± 21.2	423.3 ± 27.7
			Placebo	446.1 ± 29.5	451.8 ± 25.8	447.3 ± 33.8
Hb	g/dL	M：13.5-17.6	Salacia	14.8 ± 1.3	14.9 ± 1.6	14.8 ± 1.1
			Placebo	14.9 ± 0.4	15.1 ± 0.3	15.0 ± 0.6
		F：11.3-15.2	Salacia	12.7 ± 0.4	12.5 ± 0.6	12.3 ± 0.5
			Placebo	12.9 ± 2.0	13.0 ± 1.8	12.9 ± 2.0
Ht	%	M：39.8-51.8	Salacia	44.0 ± 2.9	43.9 ± 3.4	43.9 ± 2.2
			Placebo	44.3 ± 1.3	44.8 ± 1.3	44.7 ± 2.0
		F：33.4-44.9	Salacia	38.8 ± 1.5	38.4 ± 1.8	37.8 ± 1.6
			Placebo	39.4 ± 3.9	40.1 ± 4.2	39.3 ± 4.0
PLT	$\times 10^4/\mu L$	M：13.1-36.2	Salacia	22.0 ± 3.6	21.9 ± 3.0	22.6 ± 2.7
			Placebo	25.9 ± 5.4	25.4 ± 5.7	26.3 ± 5.4
		F：13.0-36.9	Salacia	26.2 ± 2.8	26.9 ± 2.7	27.7 ± 3.1
			Placebo	26.2 ± 3.2	27.6 ± 3.2	26.5 ± 2.7
MCV	fL	M：82.7-101.6	Salacia	90.7 ± 2.7	90.6 ± 3.1	90.5 ± 3.7
			Placebo	90.1 ± 4.5	90.2 ± 4.5	90.0 ± 3.9
		F：79.0-100.0	Salacia	89.9 ± 4.5	90.0 ± 4.8	89.5 ± 4.8
			Placebo	88.5 ± 9.8	89.1 ± 10.5	88.2 ± 10.1
MCH	pg	M：28.0-34.6	Salacia	30.5 ± 0.8	30.6 ± 1.0	30.5 ± 1.1
			Placebo	30.3 ± 1.5	30.4 ± 1.4	30.3 ± 1.6
		F：26.3-34.3	Salacia	29.5 ± 1.9	29.4 ± 2.0	29.2 ± 1.6
			Placebo	28.9 ± 4.5	28.9 ± 4.4	28.9 ± 4.5
MCHC	%	M：31.6-36.6	Salacia	33.6 ± 0.8	33.8 ± 1.1	33.7 ± 1.0
			Placebo	33.7 ± 0.7	33.7 ± 0.5	33.7 ± 0.8
		F：30.7-36.6	Salacia	32.8 ± 0.9	32.6 ± 1.5	32.7 ± 0.7
			Placebo	32.4 ± 2.2	32.4 ± 1.6	32.6 ± 2.0

Data are expressed as the mean ± SD. # $p < 0.05$, ## $p < 0.01$ in comparison with week 0 by the Bonferroni test. * $p < 0.05$ in comparison with the Placebo by Student's t-test. Salacia group, n = 16 (9 males and 7 females); Placebo group, n = 16 (8 males and 8 females). See abbreviation in **Table 9**.

Table 9. Measurements of hematological and blood biochemical parameters during the long-term intake trial (Trial B).

Item	Unit	Standard value	Group	week 0	week 4	week 8	week 12
TG	mg/dL	50-149	Salacia	126.6 ± 62.2	96.7 ± 27.1	91.2 ± 31.8	97.5 ± 51.9
			Placebo	114.5 ± 104.1	114.7 ± 65.0	99.5 ± 52.4	90.5 ± 41.6
TC	mg/dL	150-219	Salacia	201.8 ± 34.2	212.5 ± 33.4	209.1 ± 38.9	203.9 ± 33.9
			Placebo	205.4 ± 31.1	213.4 ± 24.7	213.0 ± 32.3	208.3 ± 29.0
LDL-C	mg/dL	70-139	Salacia	117.7 ± 29.9	128.1 ± 27.7 #	127.1 ± 32.3	119.9 ± 29.1
			Placebo	122.9 ± 26.8	128.0 ± 22.8	130.5 ± 28.8	126.2 ± 27.5
HDL-C	mg/dL	M：40-86	Salacia	56.9 ± 12.7	59.1 ± 14.1	56.9 ± 9.9	57.1 ± 10.1
			Placebo	60.6 ± 13.1	59.4 ± 10.3	59.5 ± 11.2	58.3 ± 14.0
		F：40-96	Salacia	73.9 ± 12.7	72.8 ± 13.5	79.8 ± 14.0	76.5 ± 15.8
			Placebo	66.6 ± 10.4	72.1 ± 15.4	72.3 ± 16.5	70.4 ± 17.3
TP	g/dL	6.7-8.3	Salacia	7.5 ± 0.4	7.4 ± 0.3	7.5 ± 0.3	7.4 ± 0.4
			Placebo	7.4 ± 0.3	7.3 ± 0.4	7.3 ± 0.4	7.3 ± 0.4
ALB	g/dL	3.8-5.2	Salacia	4.6 ± 0.3	4.5 ± 0.2	4.5 ± 0.2	4.5 ± 0.2
			Placebo	4.6 ± 0.3	4.6 ± 0.3	4.6 ± 0.3	4.5 ± 0.3
UA	mg/dL	M：3.7-7.0	Salacia	6.2 ± 1.3	6.2 ± 1.0	6.1 ± 1.3	6.2 ± 1.2
			Placebo	5.8 ± 1.3	5.7 ± 1.3	6.0 ± 1.7	6.3 ± 1.4
		F：2.5-7.0	Salacia	4.4 ± 1.1	4.3 ± 1.2	4.6 ± 1.0	4.6 ± 1.0
			Placebo	4.7 ± 0.5	4.5 ± 0.7	4.7 ± 0.7	4.7 ± 0.6
Cre	mg/dL	M：0.61-1.04	Salacia	0.807 ± 0.070	0.863 ± 0.124	0.816 ± 0.050	0.798 ± 0.069
			Placebo	0.821 ± 0.095	0.828 ± 0.109	0.838 ± 0.118	0.876 ± 0.127 #
		F：0.47-0.79	Salacia	0.580 ± 0.064	0.557 ± 0.082	0.590 ± 0.089	0.597 ± 0.088
			Placebo	0.608 ± 0.085	0.641 ± 0.103	0.636 ± 0.084	0.644 ± 0.091
BUN	mg/dL	8.0-22.0	Salacia	13.5 ± 2.7	13.5 ± 3.5	13.7 ± 2.2	13.5 ± 2.3
			Placebo	12.6 ± 3.1	14.3 ± 3.7	14.7 ± 3.2	13.5 ± 3.6
AST (GOT)	U/L	10-40	Salacia	24.8 ± 8.2	25.7 ± 9.3	22.6 ± 5.9	23.3 ± 7.7
			Placebo	21.6 ± 6.4	21.4 ± 4.1	21.1 ± 5.6	21.3 ± 6.9
ALT (GPT)	U/L	5-40	Salacia	23.1 ± 12.1	27.2 ± 15.5	24.0 ± 19.2	26.8 ± 23.0
			Placebo	21.9 ± 10.9	23.2 ± 13.7	21.8 ± 11.6	19.7 ± 10.5
γ-GTP	U/L	M：≦70	Salacia	28.7 ± 12.2	27.2 ± 14.1	28.6 ± 16.3	28.9 ± 18.2
			Placebo	32.8 ± 15.5	32.3 ± 13.3	35.8 ± 29.0	30.5 ± 13.9
		F：≦30	Salacia	26.7 ± 14.5	29.7 ± 18.8	27.0 ± 10.7	29.2 ± 12.1
			Placebo	16.9 ± 7.6	18.0 ± 8.2	17.6 ± 6.7	17.3 ± 7.5
Glucose	mg/dL	70-109	Salacia	99.6 ± 10.1	99.6 ± 9.4	91.0 ± 9.7	94.8 ± 11.5
			Placebo	99.4 ± 10.4	97.5 ± 14.5	89.5 ± 8.7 #	89.7 ± 9.7
LDH	U/L	115-245	Salacia	167.3 ± 41.3	170.7 ± 35.5	164.6 ± 38.6	162.5 ± 29.7
			Placebo	167.0 ± 26.3	165.9 ± 25.1	167.1 ± 21.9	166.8 ± 26.1
HbA1c [NGSP]	%	4.6-6.2	Salacia	5.3 ± 0.3	5.3 ± 0.4	5.3 ± 0.4	5.3 ± 0.5
			Placebo	5.3 ± 0.3	5.3 ± 0.3	5.3 ± 0.3	5.3 ± 0.3
Insulin	μIU/mL	1.84-12.2	Salacia	10.4 ± 7.5	8.6 ± 5.2	6.8 ± 7.5	8.2 ± 7.8
			Placebo	9.4 ± 6.2	7.7 ± 5.2	5.1 ± 2.5	5.3 ± 3.4
Pentosidine	μg/mL	—	Salacia	0.043 ± 0.006	—	—	0.046 ± 0.008
			Placebo	0.042 ± 0.009			0.049 ± 0.012 #
Na	mEq/L	136-147	Salacia	140.2 ± 1.2	140.3 ± 1.1	140.1 ± 1.8	140.9 ± 1.5
			Placebo	139.8 ± 1.4	139.7 ± 1.6	140.1 ± 1.0	140.3 ± 1.3
Cl	mEq/L	98-109	Salacia	103.7 ± 1.4	104.3 ± 1.9	103.9 ± 1.9	105.8 ± 1.7 ##
			Placebo	103.8 ± 1.6	104.1 ± 1.6	104.9 ± 2.0	105.4 ± 1.8 #

Item	Unit	Standard value	Group	week 0	week 4	week 8	week 12
K	mEq/L	3.6-5.0	Salacia	4.1 ± 0.3	4.2 ± 0.2	4.2 ± 0.2	4.0 ± 0.2
			Placebo	4.0 ± 0.3	4.3 ± 0.3	4.3 ± 0.2	4.1 ± 0.3
Ca	mg/dL	8.5-10.2	Salacia	9.2 ± 0.5	9.1 ± 0.6	9.3 ± 0.5	9.1 ± 0.5
			Placebo	9.4 ± 0.3	9.5 ± 0.5	9.5 ± 0.5	9.4 ± 0.4
Mg	mg/dL	1.8-2.6	Salacia	2.3 ± 0.2	2.3 ± 0.1	2.3 ± 0.2	2.2 ± 0.1
			Placebo	2.2 ± 0.2	2.3 ± 0.2	2.3 ± 0.1	2.3 ± 0.2
Fe	μg/dL	M：54-200	Salacia	124.2 ± 46.3	121.0 ± 51.1	112.3 ± 30.5	98.0 ± 35.9
			Placebo	107.9 ± 25.3	95.9 ± 40.5	106.6 ± 29.2	121.3 ± 47.7
		F：48-154	Salacia	133.9 ± 67.2	100.3 ± 43.6	120.8 ± 72.4	103.2 ± 17.7
			Placebo	65.9 ± 38.0 *	92.9 ± 68.7	98.0 ± 61.1	108.9 ± 55.1
WBC	/μL	M：3900-9800	Salacia	5767 ± 811	5722 ± 1248	6267 ± 1555	5689 ± 1852
			Placebo	5763 ± 1548	5725 ± 1442	5588 ± 1080	5713 ± 1238
		F：3500-9100	Salacia	5514 ± 1272	5150 ± 428	5550 ± 532	5383 ± 1091
			Placebo	5300 ± 1744	5529 ± 1778	5357 ± 1190	5071 ± 1259
RBC	$\times 10^4/\mu L$	M：427-570	Salacia	490.7 ± 41.0	498.6 ± 48.8	491.8 ± 42.1	485.8 ± 46.6
			Placebo	496.9 ± 34.4	499.1 ± 44.8	489.0 ± 38.4	487.3 ± 36.7
		F：376-500	Salacia	441.0 ± 22.1	436.5 ± 23.9	448.7 ± 33.4	433.5 ± 22.6
			Placebo	426.6 ± 25.9	433.1 ± 30.3	426.6 ± 27.7	426.7 ± 24.1
Hb	g/dL	M：13.5-17.6	Salacia	15.2 ± 1.1	15.4 ± 0.9	15.0 ± 1.0	14.8 ± 0.9
			Placebo	15.1 ± 0.9	15.1 ± 1.1	14.7 ± 0.9	14.7 ± 0.8
		F：11.3-15.2	Salacia	13.3 ± 0.8	13.1 ± 0.7	13.5 ± 0.7	13.0 ± 0.5
			Placebo	12.6 ± 1.3	12.5 ± 1.2	12.3 ± 1.1	12.3 ± 1.0
Ht	%	M：39.8-51.8	Salacia	44.2 ± 2.5	45.2 ± 2.7	44.2 ± 2.5	42.8 ± 2.6
			Placebo	44.3 ± 2.3	44.7 ± 2.9	43.5 ± 2.8	43.1 ± 2.3
		F：33.4-44.9	Salacia	40.4 ± 1.9	40.1 ± 1.9	41.2 ± 2.5	39.4 ± 1.6
			Placebo	37.9 ± 2.9	38.7 ± 2.6	37.4 ± 2.4 *	37.4 ± 2.5
PLT	$\times 10^4/\mu L$	M：13.1-36.2	Salacia	21.3 ± 2.3	21.2 ± 1.7	22.2 ± 2.7	21.8 ± 3.1
			Placebo	23.9 ± 4.0	25.0 ± 4.3 *	26.1 ± 5.9	24.3 ± 4.1
		F：13.0-36.9	Salacia	24.7 ± 3.4	23.9 ± 3.1	23.6 ± 3.5	24.8 ± 3.0
			Placebo	26.3 ± 2.9	26.0 ± 3.1	26.8 ± 3.2	26.5 ± 3.3
MCV	fL	M：82.7-101.6	Salacia	90.4 ± 3.7	90.9 ± 4.4	90.2 ± 4.5	88.4 ± 4.1 #
			Placebo	89.2 ± 4.0	89.8 ± 3.5	89.0 ± 3.1	88.6 ± 3.2
		F：79.0-100.0	Salacia	91.6 ± 4.7	91.9 ± 4.0	92.1 ± 4.7	91.1 ± 4.3
			Placebo	89.0 ± 5.8	89.6 ± 7.5	88.1 ± 7.8	87.8 ± 7.4
MCH	pg	M：28.0-34.6	Salacia	31.1 ± 1.4	30.9 ± 1.5	30.6 ± 1.6	30.5 ± 1.7 #
			Placebo	30.5 ± 1.5	30.3 ± 1.2	30.1 ± 1.3	30.2 ± 1.2
		F：26.3-34.3	Salacia	30.2 ± 1.9	30.2 ± 2.0	30.1 ± 1.7	30.1 ± 1.6
			Placebo	29.6 ± 2.5	29.1 ± 3.2	29.0 ± 3.1	29.0 ± 2.9
MCHC	%	M：31.6-36.6	Salacia	34.4 ± 0.6	34.0 ± 0.9	34.0 ± 0.8	34.5 ± 0.6
			Placebo	34.2 ± 0.7	33.7 ± 0.6	33.7 ± 0.4	34.0 ± 0.6
		F：30.7-36.6	Salacia	33.0 ± 0.5	32.8 ± 0.8	32.6 ± 0.5	33.1 ± 0.4
			Placebo	33.2 ± 1.0	32.3 ± 1.2 ##	32.9 ± 1.2	32.9 ± 0.9

Data are expressed as the mean ± SD. * $p < 0.05$ in comparison with the Placebo by Student's t-test. # $p < 0.05$, ## $p < 0.01$ in comparison with week 0 by the Bonferroni test. Salacia group, n = 16 (9 males and 7 females); Placebo group, n = 16 (8 males and 8 females). TG, triglyceride; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TP, total protein; ALB, albumin; UA, uric acid; Cre, creatinine; BUN, blood urea nitrogen; AST, asprtate aminotransferase; ALT, alanin aminotransferase; γ-GTP, gamma glutamyl transpeptidase; LDH, lactate dehydrogenase; HbA1c [NGSP], hemoglobinA1c [National Glycohemoglobin Standardization Program]; Na, natrium; Cl, chloride; K, potassium; Ca, calcium; Mg, magnesium; Fe, ferrum; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Ht, hematocrit; PLT, platelet; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; SD, standard deviation.

Table 10. Parameters of the urine test during the excess intake trial (Trial A).

Item	Group	week 0				week 2				week 4			
		–	+/–	1+	2+	–	+/–	1+	2+	–	+/–	1+	2+
Sugar	Salacia	16	0	0	0	16	0	0	0	16	0	0	0
	Placebo	16	0	0	0	16	0	0	0	15	0	0	0
Protein	Salacia	13	3	0	0	14	2	0	0	14	2	0	0
	Placebo	14	1	1	0	12	3	1	0	9	5	1	0
Urobilinogen	Salacia	0	16	0	0	0	16	0	0	0	16	0	0
	Placebo	0	16	0	0	0	16	0	0	0	15	0	0
Bilirubin	Salacia	16	0	0	0	16	0	0	0	16	0	0	0
	Placebo	16	0	0	0	16	0	0	0	15	0	0	0
Ketone bodies	Salacia	16	0	0	0	16	0	0	0	16	0	0	0
	Placebo	16	0	0	0	16	0	0	0	15	0	0	0

Data are expressed as the number of cases. No significant difference. Salacia group, n = 16 (9 males and 7 females); Placebo group, n = 16 (8 males and 8 females).

Table 11. Parameters of the urine test during the long-term intake trial (Trial B).

Item	Group	week 0				week 4				week 8				week 12			
		–	+/–	1+	2+	–	+/–	1+	2+	–	+/–	1+	2+	–	+/–	1+	2+
Sugar	Salacia	16	0	0	0	15	0	0	0	15	0	0	0	15	0	0	0
	Placebo	16	0	0	0	15	0	0	0	15	0	0	0	15	0	0	0
Protein	Salacia	9	7	0	0	14	1	0	0	# 14	1	0	0	# 12	3	0	0
	Placebo	14	2	0	0	15	0	0	0	14	1	0	0	14	1	0	0
Urobilinogen	Salacia	0	16	0	0	0	15	0	0	0	15	0	0	0	15	0	0
	Placebo	0	16	0	0	0	15	0	0	0	15	0	0	0	15	0	0
Bilirubin	Salacia	16	0	0	0	15	0	0	0	15	0	0	0	15	0	0	0
	Placebo	16	0	0	0	15	0	0	0	15	0	0	0	15	0	0	0
Ketone bodies	Salacia	15	0	0	1	15	0	0	0	15	0	0	0	15	0	0	0
	Placebo	16	0	0	0	15	0	0	0	15	0	0	0	14	0	1	0

Data are expressed as the number of cases. # $p < 0.05$ in comparison with week 0 by the Wilcoxon signed-rank test. Salacia group, n = 16 (9 males and 7 females); Placebo group, n = 16 (8 males and 8 females).

Trial A

No significant differences were observed.

Trial B

The number of subjects who were protein (±) in the Salacia group at week 0 was greater than that of the Placebo group ($p = 0.053$). At week 12, five protein (±) subjects in the Salacia group turned to (–) and two protein (±) subjects remained (±). No abnormal changes in qualitative levels of sugar, urobilinogen, bilirubin, or ketone bodies were observed in any subject.

Discussion

We have previously reported a double-blind crossover trial on the preventive effect of Salacia on postprandial hyperglycemia [2]. The results showed that gyudon topping containing Salacia (0.5 mg salacinol) suppressed the elevation in postprandial blood glucose levels and serum insulin levels induced by steamed rice intake. Both these diabetic parameters were more significantly suppressed in subjects whose HOMA-R (homeostatic model assessment: insulin resistance) was ≧ 1.73

In this study, to evaluate the safety of daily intake of the gyudon topping, we selected a dose of 0.6 mg salacinol, which was 0.1 mg higher than the effective dose in the previous study. The excess intake trial, using three times the regular dose, was conducted with 1.8 mg/day for 4 weeks, and the long-term intake trial was conducted with 0.6 mg/day for 12 weeks.

Safety evaluation

No adverse effects related to the test food containing Salacia were observed in the excess intake and long-term

intake trials. Physical examination as well as blood and urine analysis were performed. Several significant changes were sporadically observed within the normal range, but the magnitude of the changes were small and they were not clinically significant.

Several previous safety studies on members of the Salacia family have been reported: Ozaki *et al*. conducted an excessive intake trial with 900 mg/day S. reticulata extract [8]; Beppu *et al*. conducted a 12-week intake trial with 450 mg/day *S. reticulata* granule extract [9]. Kobayashi *et al*. conducted a five-fold excessive intake trial and a long-term intake trial using tablets containing *S. chinensis* extract [10]. Although the contents of salacinol were not reported in these papers, they were estimated to be between 0.474 mg and 0.552 mg from correlations with enzyme inhibitory activity [11]. No clinically significant effects were recorded in any of these studies. In addition, this study has confirmed that 1.8 mg/day salacinol over 4 weeks and 0.6 mg/day salacinol over 12 weeks did not cause adverse effects.

It was therefore concluded that there was no risk to safety from gyudon topping containing 0.6 mg salacinol.

Parameters related to metabolic syndrome

The fasting blood glucose levels in the Salacia group were significantly decreased in the excess intake trial at week 4, even though the values were within the normal range. The fasting blood glucose levels in the long-term intake trial were decreased, but the change was not statistically significant.

Insulin level in the Salacia group in the excess intake trial showed a time-dependent reduction ($p = 0.059$ at week 4), but not in the Placebo group ($p = 0.094$ at week 4). In contrast, in the long-term intake trial, the insulin level at week 12 was not reduced in either group in comparison with week 0. In this study, the fasting glucose level, but not the postprandial blood glucose level, was measured for safety evaluation and the long-term ingestion of a regular dose was proven not to increase the risk of hypoglycemia.

Pentosidine, one of the end products of advanced glycation, is a fluorescent substance first isolated from the human brain by Sell and Monnier [12]. The oxidation process is deeply related to its production; it is considered a marker that reflects the glycosylation and oxidation of proteins in a living body. In this study, pentosidine was significantly increased in the Placebo group, but not in the Salacia group; however, these differences were not statistically significant. Thus, the ability of the Salacia extract to suppress pentosidine production is thought to be limited.

Even in the preceding long-term intake studies with a regular dose of Salacia extract, fasting blood glucose, insulin, and HbA1c were not different when compared with the corresponding Placebo group. Other glycation markers, glycoalbumin and 1.5-AG, were investigated in two studies; effectiveness was reported in one study, but not in the other. Pentosidine has not been previously evaluated.

The results of this study, combined with previous studies, indicated that Salacia did not have a huge influence on carbohydrate metabolism. Therefore, gyudon toppings containing a regular dose of Salacia extract can be considered safe.

In the excess intake trial of Salacia, the 1.8 mg/day salacinol dose used in this study may be effective for the treatment of obesity and hypertension. Body fat and diastolic pressure at the end of the excess intake trial period in the Salacia group were significantly decreased in comparison with week 0. In contrast, the long-term intake trial showed only a slight decline, which was not significant between the Salacia and Placebo groups.

S. reticulata has been reported to improve obesity and associated metabolic disorders in TSOD mice [13]. A supplement of 1% *S. reticulata* extract lowered the accumulation of fat and blood pressure after 8 weeks. Consistent results on the changes in blood pressure and body fat after Salacia ingestion have not been obtained; therefore, further research is required.

The presence of diabetes generally leads to an increase in salt sensitivity, which is accompanied by high blood pressure [14]. Diabetic nephropathy, a typical complication of diabetes, increases the frequency of complications of hypertension as it progresses. Patients with diabetes are prone to water loss and have a tendency to accumulate salt. The mechanism by which hyperglycemia induces polyuria occurs through the enhanced function of the renin–angiotensin–aldosterone system (RAS) and the increase in sodium reabsorption to prevent sodium loss. Increased RAS function is a direct cause of hypertension. In addition, recent studies have reported that glycation stress is involved in the diabetes mellitus-mediated increase in salt sensitivity [15]. Methylglyoxal is an oxidative stress marker that is found elevated in patients with chronic renal failure. The increase in blood pressure in salt-sensitive rats was higher after the administration of salt alone; Methylglyoxal also induced albuminuria, glomerular sclerosis, and renal tubular damage, indicating that glycation stress enhances salt sensitivity due to diabetes.

The dietary approaches to stop hypertension (DASH) diet has been used to assist in the reduction of sodium intake and fat intake and successfully lower blood pressure [16].

The reduction of the salt and fat in the both Placebo and Salacia groups appeared to be equally effective at the regular dose level. The amounts of sodium (as the salt equivalent) and fat in the test food corresponded to approximately 22% and 31%, respectively, of the recommended daily intake for 30–59-year-old men. Foods that contain reduced salt and fat, such as the test food, are also preferable from the perspective of hypertension prevention.

We have provided comprehensive evidence that gyudon topping containing a Salacia extract is a safe product for the prevention of postprandial hyperglycemia and can offer a rich diet to assist the maintenance of health of individuals with prediabetes.

Conclusions

The safety of gyudon topping containing a Salacia extract was confirmed in an excess intake trial and a long-term intake trial. Consistent with previous data, Salacia-containing foods ameliorate postprandial hyperglycemia, and may contribute to the prevention of diabetes.

Declaration of Conflict of Interest

This research received support from Yoshinoya Holdings.

References

1) Ogura M, Takabe W, Nagano Y, et al. Physical effects of 12-week protocol of daily gyudon (beef bowl) ingredients consumption. Glycative Stress Res. 2016; 3: 99-109.
2) Kajiwara N, Onodera K, Tsuji T, et al. A study for evaluating the effect of the intake of meal containing Salacia extract on postprandial hyperglycemia. Glycative Stress Res. 2017; 4: 117-123.
3) Kishino E, Ito T, Fujita K, et al. A mixture of *Salacia reticulata* (Kotala himbutu) aqueous extract and cyclodextrin reduces body weight gain, visceral fat accumulation, and total cholesterol and insulin increases in male Wistar fatty rats. Nutr Res. 2009; 29: 55-63.
4) Yoshikawa M, Morikawa T, Matsuda H, et al. Absolute stereostructure of potent α-glucosidase inhibitor, Salacinol, with unique thiosugar sulfonium sulfate inner salt structure from *Salacia reticulata*. Bioorg Med Chem. 2002; 10: 1547-1554.
5) Morikawa T, Akaki J, Ninomiya K, et al. Salacinol and related analogs: New leads for type 2 diabetes therapeutic candidates from the Thai traditional natural medicine *Salacia chinensis*. Nutrients. 2015; 7: 1480-1493.
6) Shimada H, Asano I, Yamada Y. Antigenicity and phototoxicity of water-soluble extract from *Salacia reticulata* (Celastraceae). Food Hygiene and Safety Science (Shokuhin Eiseigaku Zasshi). 2001; 42: 144-147. (in Japanese)
7) Shimada H, Furuhashi T, Naitou K, et al. Thirteen-week repeat dose oral toxicity study of *Salacia reticulata* extract in rats. Japanese Journal of Medicine and Pharmaceutical Science (Igaku to Yakugaku). 2001; 46: 527-540. (in Japanese)
8) Ozaki S, Tamura H, Kataoka K, Effects of repeated overdoses of Kothala-himbutu extract in drinks. Nippon Shokuhin Kagaku Kogaku Kaishi. 2008; 55: 481-486. (in Japanese)
9) Beppu H, Shikano M, Fujita K, et al. Effects of single and 3-month repeated ingestions of a Kothalahim granule preparation containing Kothala himbutu extract on the glucose metabolism of humans. Journal of Japanese Council for Advanced Food Ingredients Research. 2005; 8: 105-117 (in Japanese)
10) Kobayashi M, Akagi J, Yamaguchi Y, et al. Safety evaluation of long-term and excess intake of the tablet containing hot water extract of *Salacia chinensis*. Japanese Pharmacology & Therapeutics. 2016; 44: 399-408. (in Japanese)
11) Japan Anti-Aging Association, Data book of functional foods, Medical Review, 2016. (in Japanese)
12) Sell DR, Monnier VM. Structure elucidation of senescence cross-link from human extracellular matrix. J Biol Chem. 1989; 264: 21597-21602.
13) Akase T, Shimada T, Harasawa Y, et al. Preventive effects of *Salacia reticulata* on obesity and metabolic disorders in TSOD mice. Evid Based Complement Alternat Med. 2011; 2011: 484590-484600.
14) Motokawa M, Kimura G. Salt restriction for treatment of diabetes mellitus. Nippon Rinsho. 2005; 63: 417-423. (in Japanese)
15) Chen X, Mori T, Guo Q, et al. Carbonyl stress induces hypertension and cardio-renal vascular injury in Dahl salt-sensitive rats. Hypertens Res. 2013; 36: 361-367.
16) Sacks F, Svetkey L, Vollmer W, et al. Effects on blood pressure of reduced dietary sodium and the dietary approaches to stop hypertension (DASH) diet. N Engl J Med. 2001; 344: 3-10.