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Safety evaluation of excessive intake of Hesperetin-7-Glucoside-β-Cyclodextrin inclusion complex in healthy Japanese subjects

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Submission Date: December 29th, 2023; **Acceptance Date:** February 24th, 2024; **Publication Date:** March 4th, 2024

Please cite this article as: Moriwaki M., Abe A., Kapoor M. P., Yamaguchi A., Okamoto S., Ozeki. Safety evaluation of excessive intake of Hesperetin-7-Glucoside-β-Cyclodextrin Inclusion Complex in Healthy Japanese Subjects. *Functional Foods in Health and Disease* 2024; 14(3): 157-168. DOI: https://doi.org/10.31989/ffhd.v14i3.1325

ABSTRACT

Background: Hesperidin, a flavonoid glycoside, is widely found in the peels and rinds of citrus fruits, offering various physiological benefits. However, its effectiveness is hindered by challenges related to insolubility and low bioavailability. To overcome these obstacles, we developed the hesperetin-7-glucoside -β-cyclodextrin inclusion complex (HCD). This complex demonstrates superior solubility and bioavailability compared to hesperidin. In a previous study where participants consumed 300 mg/day of HCD for 12 weeks, there was a notable improvement in endothelial dysfunction. Importantly, no significant adverse clinical events were reported during this period.

Objective: To evaluate the safety of the excessive intake of HCD in healthy Japanese subjects.

Methods: Fourteen healthy male and female volunteers (with a mean age of 39.1±9.1) participated in this excessive HCD intake clinical trial. Subjects took 1500 mg/day HCD, which was five times the dosage of 300 mg/day HCD, for 4 consecutive weeks. Physical examination, blood tests, and urin tests were performed during this period.

Results: Results demonstrated no significant differences at 2, and 4 weeks compared to the baseline at 0 weeks with 1500 mg HCD (equivalent to 195 mg HPTG) supplementation in healthy subjects.

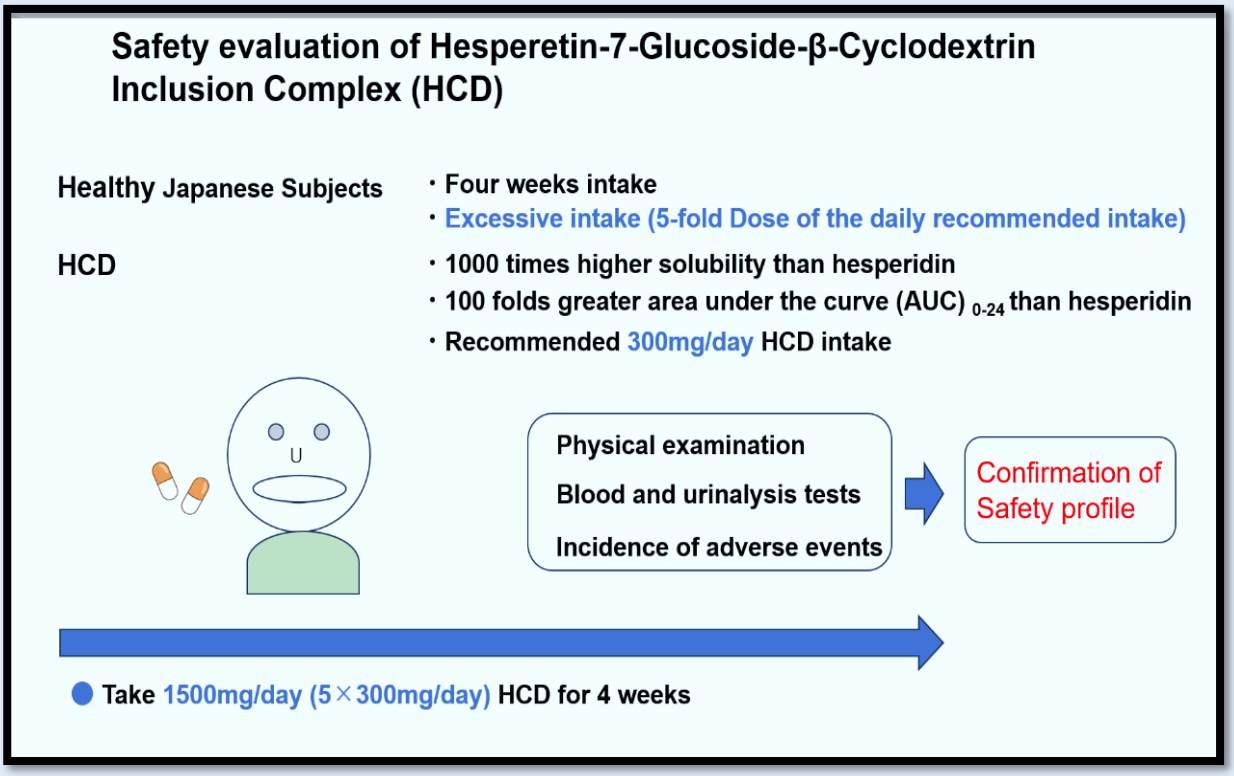
Conclusions: It has been demonstrated that there are no safety concerns when consuming 1500 mg of HCD daily,

continuously for 4 weeks.

Keywords: Safety, Bioavailability, Clinical trials, Hesperidin, Food, Foods with Function Claims, Overdose supplementation, Cyclodextrin

Trial registration: UMIN-CTR (Trial ID: UMIN000051960)

Foundation: Taiyo Kagaku Co. Ltd.



INTRODUCTION

Consumers are progressively exploring dietary strategies to prevent and manage health conditions, recognizing that a balanced diet enriched with functional foods can greatly enhance their overall health and lifestyle [1].

In the USA, the Functional Food Center (FFC) has promoted a multi-step procedure for the development and marketing of functional food products, eliminating the need to classify them as conventional items. In 2021, FFC put forth a proposed definition of the functional food as the following: “Natural or processed foods that contain biologically active compounds, which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms” [2].

In Japan, the Foods with Health Claims (FHC) system

was established in April 2001 to provide consumers with products backed by scientific evidence. This system guarantees the safety and efficacy of foods with functional claims. It mandates that sellers submit comprehensive dossiers to the Consumer Affairs Agency (CAA). The required data for foods with function claims includes not only evidence of effectiveness but also proof of safety levels. Once the necessary documents have been submitted and reviewed by the CAA, the product can then be marketed [2].

Hesperidin (HSP; CAS 520-26-3), known as hesperetin (HPT; CAS 520-33-2)-7-rutinoside (where rutinoside includes glucose and rhamnose), is a flavonoid glycoside. It is found in abundance in the peels and rinds of citrus fruits such as sweet oranges (*Citrus sinensis*), grapefruits *(Citrus paradise*), tangerines (*Citrus reticulata*), limes (*Citrus aurantifolia*), and lemons (Citrus limon) [4-5].

Past research has shown that taking HSP at a dosage of 500 to 1000mg daily for a period of 3 to 12 weeks can lead to various physiological benefits. These include improvements in cardiovascular disease risk, metabolic syndrome, endothelial function, diabetes management, and inflammatory properties [6-13]. However, the use of HSP poses a challenge due to its poor solubility and low bioavailability.

To overcome these challenges, some researchers have shown that the solubility and bioavailability of insoluble flavonoids like HSP and HPT could potentially be improved by forming complexes with cyclodextrin (CD) [11-13]. In addition, flavonoid mono-glucosides (FMGs), exemplified by HPTG, naringenin-7-glucoside, and others, have demonstrated absorption primarily in the small intestine rather than the large intestine. This phenomenon has led to a notable increase in their bioavailability [17-19].

Therefore, we developed the HPT-7-glucoside (HPTG)-β-CD inclusion complex (HCD) using a patented method [20-21]. This method involves treating HSP with naringinase (CAS 9068-31-9) in the presence of β-CD (CAS 7585-39-9). Our findings showed that the solubility of HCD was over 1000 times greater than that of HSP. Furthermore, the area under the curve (AUC) $_{0\text{-}24}$ of the total plasma HPT concentration after consuming 1.05g HCD was over 100 times greater than that after consuming 1.05g HSP/dextrin. In this single administration, no adverse significant events were observed during this study [22].

Before subjects were administered the substance continuously, a subchronic toxicity assessment was performed on rats. The assessment revealed that the No-Observed-Adverse-Effect Level (NOAEL) of HCD was 3267.7 mg/kg/day, which is equivalent to 464 mg of HPTG for males, and 3652.4 mg/kg/day, equivalent to 519 mg of HPTG for females [23]. The NOAEL data indicates that the acceptable daily intake (ADI) for HCD in humans can be determined as 32.7 mg HCD/kg body weight/day. Alternatively, it can be expressed as 2287 mg HCD/person/day, assuming an average human body weight of 70 kg.

In a randomized, parallel, double-blind, and placebo-controlled study involving 59 healthy individuals, it was found that the intake of 300mg HCD, containing 39 mg of HPTG (equivalent to a reduced dosage of 51mg HSP, compared to the typical 500-1000 mg HSP), resulted in a significant improvement in endothelial dysfunction, as indicated by the increase in Flow-mediated Vasodilation (FMD) % over 12 weeks, in comparison to the placebo group. Importantly, no events of clinical relevance were noted during this time [24].

According to the revised notification of foods with functional claims by the Consumer Affairs Agency (CAA)

in Japan (September 2023), functional foods in Japan must undergo evaluation for both their scientific effects and safety. In clinical trials, the long-term safety of food is assessed over a period of 12 weeks. Furthermore, the quantity of functional ingredients included in the daily intake of the final product is carefully examined to prevent potential health risks, especially when the quantity exceeds the amount of the ingredient. For processed foods in supplement form, the intake amount is increased fivefold, while for other processed foods and fresh foods, the intake amount is tripled higher than the recommended daily intake over a span of 4 weeks. These precautions are taken to ensure the safety and effectiveness of functional foods in Japan [25].

Therefore, a comprehensive safety assessment was undertaken involving 14 healthy adults (comprising 5 males and 9 females) with an average age of 39.1±9.1. Over a 4-week period, participants were administered a daily total of 1500 mg of HCD, equivalent to 195 mg of HPTG and falling below the acceptable daily intake (ADI) of HCD. The dosage was distributed into 5 capsules after breakfast and 5 capsules after dinner. It is noteworthy that this dosage represents a fivefold increase over the recommended daily intake of 300 mg HCD.

MATERIALS AND METHODS

Ethical approval of the study protocol: On August 18, 2023, the Seishukai Medical Corporation Seishukai Clinic's institutional review board approved the study protocol, which was registered with the UMIN-CTR under the Trial ID: UMIN000051960. The protocol remained consistent from the final setup and throughout the study. The study was conducted under the supervision of a physician according to the 'Helsinki Declaration' principles (revised at the WMA Fortaleza General Assembly in Brazil in October 2013). It also adhered to the 'Ethical Guidelines for Life Science and Medical Research Involving Humans in Japan', established on March 23, 2021, and partially revised on March 10, 2022.

Supplementation and sample size: Capsules containing 150 mg of HCD per capsule (SUNACTIVE® HCD/HES, Taiyo Kagaku Co., Ltd., Japan) were prepared for this study. The dosage administered was 1500 mg of HCD daily, equivalent to 195 mg of HPTG. This dosage was divided into two dosages: 5 capsules after breakfast and 5 capsules after dinner. This dosage is five times the recommended dosage of 300 mg of HCD per 2 capsules (equivalent to 39.0mg of HPTG) per day. The duration of this study was 4 weeks.

Table 1. Analysis of the nutrient composition of the test food (/100g)

Protein	g	0.4
Fat	g	0.5
Carbohydrate	g	84
Energy	kcal	342
Fiber	g	-
Salt equivalent	g	9.15

Study Participants: Healthy adult males and females were recruited as volunteer participants in this study. All individuals received detailed explanatory documents and consent forms, and the study's purpose and protocol procedures were thoroughly explained. The inclusion criteria for participant selection were as follows:

(1) Healthy subjects, males and females, aged 20 or over, with no limit.

(2) Individuals who had been thoroughly briefed on the objectives and details of the research, possessed a comprehensive understanding, and willingly indicated their intention to participate could furnish written consent to be part of the study.

The following exclusion criteria were applied in this study:

(**1**) Subjects currently undergoing medical treatment or therapy, including medication and traditional herbal medicine, for any form of illness or condition.

(**2**) Subjects undergoing dietary and exercise therapy under the supervision of a doctor

(**3**) Subjects with a current or past history of severe medical conditions.

(**4**) Subjects who regularly consume over-the-counter pharmaceuticals, quasi-drugs, supplements, specific health-use foods, and foods with function claims. (Those who can stop taking them after obtaining consent are eligible to participate.)

(**5**) Subjects with a current or past history of medication allergies or food allergies

(**6**) Subjects who have donated blood components or 200 ml of whole blood within 1 month from the start of this study

(**7**) Participants who have contributed 400 ml of whole blood within the initial 4 months of this study.

(**8**) Subjects who have participated or are currently participating in other clinical trials or clinical research within one month before obtaining consent, or those who plan to participate during the study period

(**9**) Currently pregnant or breastfeeding subjects, or those who are planning to become pregnant during the study period.

(**10**) Subject who regularly drinks a lot of alcohol (equivalent to 60 g/day in terms of alcohol content

(**11**) Subjects with extremely irregular eating habits, shift workers, and subjects with an irregular life rhythm, such as those working night shifts

(**12**) Subjects who are judged by the principal investigator to be unsuitable for participation in this study.

Study design: The study protocol's systematic flow chart, single-arm trial is shown in Figure 1. Out of 27 potential participants, 14 subjects (n = 14) were chosen based on the established inclusion/exclusion criteria. Notably, there were no dropouts during the study, and all 14 subjects completed the study, contributing to the safety analysis.

The baseline characteristics of study subjects (n=14, Male/Female: 5/9) are shown in Table 2. The responsible physician, taking into account the results of previous

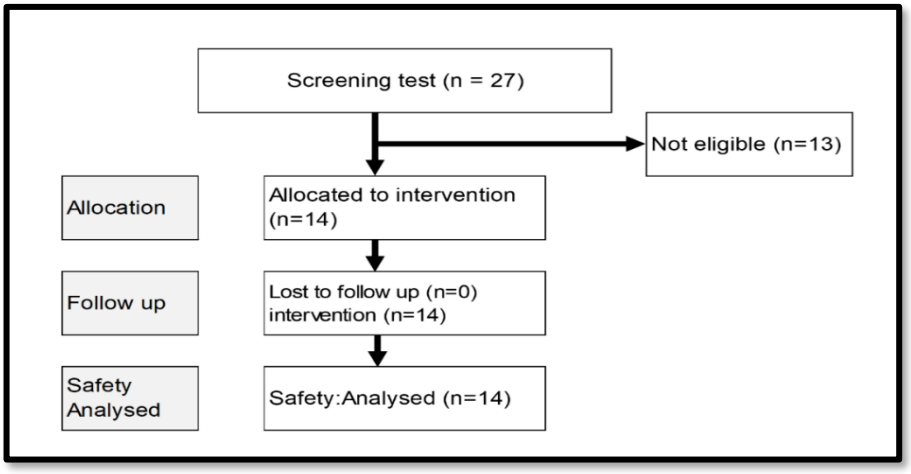


Figure 1. Systematic flow chart of this study

clinical trials determined that there was no clinical problem with the safety of the overdose test, by statistically evaluating it, with 14 healthy subjects who were selected.

Schedule and outcomes: The study was carried out at the Seishukai Medical Corporation Seishukai Clinic in Tokyo, Japan, from August 22 to September 28, 2023. The clinic was responsible for the allocation of subjects, and all relevant information was kept confidential from both the subjects and investigators until the intervention study was completed.

Safety assessments were performed using plasma hematological and biochemical tests, as well as urine tests, at three different intervals: before intake (0 weeks), at two weeks, and at 4 weeks after intake. Alongside these tests, vital signs such as blood pressure and pulse rate, as well as weight and BMI were monitored. Medical interviews were also conducted at these same intervals. Furthermore, participants were asked to keep a daily logbook for the duration of the study.

Table 2. Baseline characteristics of study subjects (n=14, Male/Female:5/9)

Parameter	Unit	Standard value	Mean		SD
Age	years	-	39.10	±	9.20
Weight	kg	-	55.23	±	8.27
BMI	kg/m^2	18.5 ~ 25	20.96	±	2.43
Systolic blood pressure	mmHg	90 ~ 139	119.57	±	8.40
Diastolic blood pressure	mmHg	40 ~ 89	71.64	±	7.04
Pulse rate (bpm)	bpm	40 ~ 100	74.71	±	10.03

Data are expressed as the mean ± SD.

Statistical analysis: Physical examination, blood analysis, and urine tests (pH, WBC) were expressed as mean and standard deviation (SD) and were analyzed $^{※}p < 0.05$, $^{※※}p < 0.01$ in comparison with 0 w using the paired t-test. Urine tests (protein, sugar, occult blood test, bilirubin, and ketone bodies) are calculated as conversion as follows: -=0,±=1,1+=2,2＋=3,3＋=4,4＋ = 5, and urobilinogen is calculated as conversion as follows: ±=0,1+=1,2＋=2,3＋=3,4＋=4 and were analyzed $^{※}p < 0.05$, $^{※※}p < 0.01$ in comparison with 0 w by the Wilcoxon signed-rank test.

RESULTS AND DISCUSSION

Intake status of test food: The intake rate of the test food was 100% for each subject.

Body measurement and physical examination: The changes in body measurements and physical examinations during the test period are presented in Table 3. When compared to the baseline at 0 weeks, there was a significant decrease in weight after 4 weeks of consumption. However, BMI did not show a significant decrease and remained within the normal range, leading the responsible physician to conclude that there were no

clinical issues. Additionally, there were no significant differences observed in systolic blood pressure, diastolic blood pressure, or pulse rate.

Table 3. Measurements of physical parameters during the excess intake trial

Item	Unit	Standard value	Week	0 week			*p*-Value
Weight	kg	-	0 week	55.23	±	8.27	-
			2 weeks	55.45	±	8.15	0.508
			4 weeks	54.72	±	7.97	0.040※
BMI	kg/m^2	18.5～25	0 week	20.96	±	2.43	-
			2 weeks	21.08	±	2.62	0.371
			4 weeks	20.79	±	2.48	0.056
Systolic blood pressure	mmHg	90～139	0 week	119.57	±	8.40	-
			2 weeks	115.86	±	9.07	0.142
			4 weeks	113.21	±	10.37	0.075
Item	**Unit**	**Standard value**	**Week**	**0 week**			***p*-Value**
Diastolic blood pressure	mmHg	40～89	0 week	71.64	±	7.04	-
			2 weeks	70.71	±	9.39	0.746
			4 weeks	70.29	±	9.55	0.620
Pulse rate	bpm	40～100	0 week	74.71	±	10.03	-
			2 weeks	73.21	±	9.64	0.441
			4 weeks	74.57	±	8.77	0.962

※$p < 0.05$, ※※$p < 0.01$ in comparison with 0 week by the paired t-test.

Blood tests: During the examination period, blood tests were conducted, the results of which are presented in Tables 4 and 5. These tests revealed no significant difference in hematological values when compared to the baseline at 0 weeks, as shown in Table 4. However, in the biochemical analysis of the blood, there was a significant increase in the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) associated with liver function damage after 4 weeks of consumption, as shown in Table 5. Despite these increases, all variations remained within the standard range. Consequently, the examining physician has determined these variations to be clinically insignificant. Moreover, there were no substantial alterations in the levels of LDH-cho, ALP, T-Bil, HDL-cho, TG, and ALB, which are linked to liver function impairment, at 2 and 4 weeks in the plasma biochemical parameters when compared to the baseline at 0 weeks, and these levels consistently stayed within the standard range [26-28].

Table 4. Measurements of hematological parameters

Item	Unit	Standard value	Week	Mean		SD	*p*-Value
RBC	$\times 10^4/\mu L$	M=438～577	0 weeks	448.43	±	38.21	-
		F=376～516	2 weeks	455.14	±	42.24	0.374
			4 weeks	456.14	±	42.13	0.291
WBC	/μL	3500～9700	0 weeks	5290.71	±	1043.46	-
			2 weeks	5297.86	±	749.86	0.976
			4 weeks	5751.43	±	1642.17	0.350
Hb	g/dL	M=13.6～18.3	0 weeks	13.20	±	1.55	-
		F=11.2～15.29	2 weeks	13.38	±	1.24	0.466
			4 weeks	13.54	±	1.09	0.238
Ht	%	M=40.4～51.9	0 weeks	40.19	±	3.71	-
		F=34.3～45.2	2 weeks	41.01	±	2.86	0.301
			4 weeks	41.07	±	2.71	0.307
MCV	fL	M＝83～101	0 weeks	89.79	±	5.90	-
		F＝80～101	2 weeks	90.50	±	5.11	0.174
			4 weeks	90.36	±	4.22	0.414
MCH	pg	M＝28.2～34.7	0 weeks	29.45	±	2.54	-
		F＝26.4～34.3	2 weeks	29.47	±	2.17	0.896
			4 weeks	29.76	±	1.76	0.223
Item	**Unit**	**Standard value**	**Week**	**Mean**		**SD**	***p*-Value**
MCHC	%	M=31.8～36.4	0 weeks	32.77	±	1.08	-
		F＝31.3～36.1	2 weekss	32.59	±	1.14	0.294
			4 week	32.94	±	0.90	0.317
PLT	$\times 10^4/\mu L$	14.0～37.9	0 weeks	24.67	±	3.87	-
			2 weeks	24.63	±	4.15	0.960
			4 weeks	24.28	±	2.77	0.530

RBC, red blood cell; WBC, white blood cell; Hb, hemoglobin; Ht, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet; SD, standard deviation.

Table 5. Measurements of blood biochemical parameters

Item	Unit	Standard value	Week	Mean		SD	*p*-Value
TP	g/dL	6.5～8.2	0 weeks	7.66	±	0.36	-
			2 weeks	7.73	±	0.45	0.622
			4 weeks	7.61	±	0.39	0.638
TG	mg/dL	50～149	0 weeks	78.29	±	31.17	-
			2 weeks	70.71	±	36.61	0.150
			4 weeks	71.93	±	39.53	0.277
T-cho	mg/dL	150～219	0 weeks	206.07	±	35.13	-
			2 weeks	212.36	±	29.59	0.175
			4 weeks	214.14	±	33.77	0.086
LDL-cho	mg/dL	70～139	0 weeks	114.64	±	23.65	-
			2 weeks	121.64	±	20.60	0.117
			4 weeks	122.86	±	25.14	0.088
HDL-cho	mg/dL	M=40～80 F=40～90	0 weeks	70.50	±	24.45	-
			2 weeks	70.79	±	11.67	0.954
			4 weeks	73.50	±	14.32	0.516
non-HDL-cho	mg/dL	-	0 weeks	135.43	±	27.41	-
			2 weeks	135.79	±	24.88	0.919
			4 weeks	135.29	±	28.02	0.967
HbA1c	%	4.6～6.2	0 weeks	5.50	±	0.27	-
			2 weeks	5.35	±	0.34	0.087
			4 weeks	5.41	±	0.38	0.406
BS	mg/dL	70～109	0 weeks	92.71	±	5.94	-
			2 weeks	92.21	±	6.04	0.652
			4 weeks	91.00	±	9.54	0.477
AST	U/L	10～40	0 weeks	19.79	±	3.14	-
			2 weeks	19.93	±	3.89	0.858
			4 weeks	21.21	±	3.75	0.042※
ALT	U/L	5～45	0 weeks	16.50	±	6.30	-
			2 weeks	17.21	±	5.67	0.528
			4 weeks	19.50	±	7.68	0.008※※

Item	Unit	Standard value	Week	Mean		SD	*p*-Value
γ-GTP	U/L	M: below 79 F: below 48	0 weeks	29.64	±	25.04	-
			2 weeks	28.29	±	22.45	0.660
			4 weeks	30.00	±	25.39	0.902
LDH	U/L	120～245	0 weeks	167.50	±	20.55	-
			2 weeks	167.50	±	20.01	1.000
			4 weeks	173.93	±	22.21	0.084
ALP	U/L	38～113	0 weeks	68.79	±	22.67	-
			2 weeks	65.29	±	20.28	0.054
			4 weeks	65.86	±	23.10	0.099
UA	mg/dL	M=3.6～7.0 F=2.7～7.0	0 weeks	4.85	±	1.11	-
			2 weeks	4.79	±	1.29	0.685
			4 weeks	5.03	±	1.10	0.269
BUN	mg/dL	8.0～20.0	0 weeks	13.17	±	4.51	-
			2 weeks	13.96	±	4.46	0.235
			4 weeks	13.19	±	4.16	0.976
T-Bil	mg/dL	0.3～1.2	0 weeks	0.69	±	0.23	-
			2 weeks	0.76	±	0.28	0.136
			4 weeks	0.81	±	0.38	0.165
ALB	g/dL	3.8~5.2	0 weeks	4.69	±	0.31	-
			2 weeks	4.71	±	0.26	0.862
			4 weeks	4.76	±	0.21	0.288
CRE	mg/dL	M=0.65～1.09 F=0.46～0.82	0 weeks	0.66	±	0.15	-
			2 weeks	0.67	±	0.15	0.765
			4 weeks	0.68	±	0.14	0.132
CK	U/L	M=50～230 F=50～210	0 weeks	107.00	±	40.32	-
			2 weeks	108.21	±	42.17	0.917
			4 weeks	112.64	±	46.97	0.682

※$p < 0.05$, ※※$p < 0.01$ in comparison with 0 week by the paired t-test.

TP, total protein; TG, triglyceride; T-cho, total cholesterol; LDL-cho, low density lipoprotein cholesterol; HDL-cho, high density lipoprotein cholesterol; non-HDL-cho, non-high density lipoprotein cholesterol; HbA1c [NGSP], hemoglobinA1c [National Glycohemoglobin Standardization Program]; BS: blood sugar; AST, aspartate aminotransferase; ALT, alanin aminotransferase; γ-GTP, gamma glutamyl transpeptidase; LDH, lactate dehydrogenase; ALP: alkaline phosphatase; ALT, alanin aminotransferase; γ-GTP, gamma glutamyl transpeptidase; LDH, lactate dehydrogenase; UA, uric acid; BUN, blood urea nitrogen; T-Bil, total bilirubin; ALB, albumin; CRE, creatinine; CK, creatine kinase

Urine tests: Table 6 shows the changes in urine tests during the study period. No significant difference was confirmed in the urine test compared to the baseline at 0 week.

Table 6. Parameters of the urine test

Item	Standard value	Weeks	Mean		SD	*p*-Value
pH	4.8-7.5	0 weeks	6.14	±	0.50	-
		2 weeks	6.11	±	0.45	0.850
		4 weeks	6.18	±	0.37	0.856
WBC	1.008-1.034	0 weeks	1.02	±	0.01	-
		2 weeks	1.02	±	0.00	0.664
		4 weeks	1.02	±	0.01	0.555
Protein	(-) ~ (±)	0 weeks	0.29	±	1.07	-
		2 weeks	0.00	±	0.00	0.317
		4 weeks	0.07	±	0.27	0.655
Sugar	(-) ~ (±)	0 weeks	0.07	±	0.27	-
		2 weeks	0.00	±	0.00	0.317
		4 weeks	0.00	±	0.00	0.317
Urobilinogen	(±)	0 weeks	0.14	±	0.53	-
		2 weeks	0.00	±	0.00	0.317
		4 weeks	0.07	±	0.27	0.655
Occult blood test	(-)	0 weeks	0.71	±	1.27	-
		2 weeks	0.71	±	1.07	0.916
		4 weeks	0.57	±	0.76	0.671
Bilirubin	(-)	0 weeks	0.21	±	0.80	-
		2 weeks	0.00	±	0.00	0.317
		4 weeks	0.00	±	0.00	0.317
Ketone bodies	(-)	0 weeks	0.21	±	0.43	-
		2 weeks	0.00	±	0.00	0.083
		4 weeks	0.14	±	0.36	0.317

※$p < 0.05$, ※※$p < 0.01$ in comparison with 0 week by the paired t-test.

WBC, white blood count. Protein, sugar, occult blood test, bilirubin, and ketone bodies are calculated as conversion as follows; -=0,±=1,1+=2,2＋=3,3＋=4,4＋ = 5 and urobilnogen is calculated as conversion as follows; ±=0,1+=1,2＋=2,3＋=3,4＋=4, and ※$p < 0.05$, ※※$p < 0.01$ in comparison with 0 week by the Wilcoxon signed-rank test.

Adverse events: The test-responsible physician confirmed that during the test period, no adverse events were observed, and the incidence of adverse events and side effects was 0%.

CONCLUSION: The safety of consuming 1500 mg of

hesperetin-7-glucoside (HPTG)-cyclodextrin (CD) inclusion compound (HCD) per day (containing 195 mg of HPTG) for 4 weeks, was verified in a study involving 14 healthy adult men and women.

The result is as follows:

1. No variations in clinical significance were observed in physical examinations, blood tests, or urine tests that could be attributed to the test food.
2. There were no reported adverse events linked to the consumption of the test food.

It has been demonstrated that there are no safety concerns when consuming five times the daily recommended intake of HCD (300 mg of HCD, which includes 39.0 mg of HPTG, per 2 capsules per day), i.e., 1500 mg of HCD (which includes 119.5 mg of HPTG) per 10 capsules per day, continuously for 4 weeks.

List of abbreviations: CAA: Consumer Affairs Agency; BMI: body mass index; HSP: hesperidin; HPTG: hesperetin-7-glucoside; CD: cyclodextrin; FMG: flavonoid-monoglucoside; AUC: area under the concentration-time curve; HCD: hesperetin-7-glucoside-β-cyclodextrin inclusion complex; NOAEL: no-observed-adverse-effect level; ADI: acceptable daily intake; BMI: body mass index; AST: aspartate aminotransferase; ALT alanine aminotransferase; γ-GTP: gamma-glutamyl transpeptidase; ALP: Alkaline phosphatase; T-Bil: Total bilirubin

Acknowledgments: The authors would like to thank Dr. Mamoru Oki (M.D) of Seishukai Medical Corporation Seishukai Clinic for his valuable medical advice as a medical practitioner for this study. The authors would also like to thank the colleagues at Taiyo Kagaku Co. Ltd., Japan, and BHN, Ltd., Japan, for their kind support during the study.

Conflict of interest statement: Taiyo Kagaku Co., Ltd. provided the funding for this study. The authors declare that there are no known competing financial interests or personal relationships that could have appeared to influence this work.

Ethical statements: The study was conducted by the Helsinki Declaration (revised during the WMA Fortaleza General Assembly in Brazil in October 2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects in Japan (Notification by the Ministry of Health, Labor, and Welfare, partially revised on February 28, 2017).

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