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Safety evaluation of high bioavailability curcumin in healthy Japanese adults: A randomized, placebo-controlled, double-blind, parallel-group comparison study

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ABSTRACT

Background: Curcumin is the principal component responsible for the pharmacological action of Curcuma longa. It has been proven to exhibit a diverse range of functions. It has been used in many fields as a spice, coloring agent, cosmetic, and food preservative.

Objective: To evaluate the safety of the intake of highly bioavailable curcumin (CR-033P and TS-P1) in humans.

Methods: We conducted two trials. The participants were healthy Japanese adults. Participants of tria1 1 (Long-term intake trial) took CR-033P or TS-P1 for 12 weeks (as curcumin 150 mg/day). Participants of Trial 2 (Excessive intake trial) took TS-P1 for 4 weeks (as curcumin 750 mg/day). The safety assessment involved monitoring the occurrence of side effects or adverse events, along with the analysis of urinalysis and blood parameters.

Results: The safety analysis population of Trial 1 included 33 participants in the CR-033P group, 32 participants TS-P1,

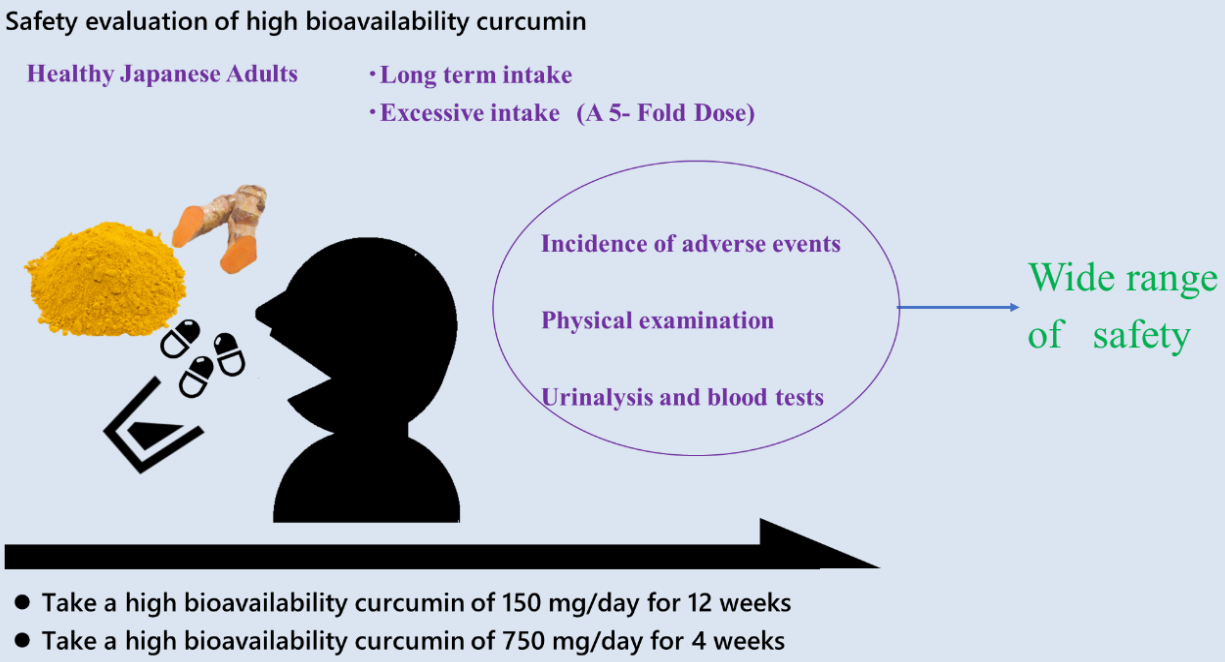
and 30 participants in the Placebo group. The safety analysis population of trial 2 included 22 participants in TS-P1 and 20 participants in the Placebo group.

In both Trial 1 and Trial 2, few participants were observed to experience adverse events and however these were not adverse events related to the CR-033P or TS-P1. Results of urinalysis and blood analysis were confirmed to not exhibit medically problematic changes related to the CR-033P or TS-P1.

Conclusions: These trials proved the safety of the long-term intake of CR-033P or TS-PI- (as curcumin 150mg/ day) and the safety of the excessive intake of TS- P1 for four weeks (as curcumin 750mg/ day). TS-P1 and CR-033P can be considered a safe curcumin supplement based on these results.

Keywords: Curcumin, bioavailability, safety, high dose, long term dose, Healthy Japanese Adults, BMI, Blood pressure

Trial registration: Trial 1: UMIN000046160, Trial 2: UMIN000048797. **Foundation:** Theravalues Corporation



INTRODUCTION

Curcuma longa has long garnered significant attention for its pharmacological effects. Not only used as a spice, coloring agent, cosmetic, and food preservative, it has also been utilized for medicinal purposes in India, China, and the rest of South-east Asia [1]. Curcumin, the principal component responsible for the pharmacological action of Curcuma longa, has been proven to exhibit a diverse range of functions. These manifest in hypothermic and hypotensive effects, while also performing roles such as being an anticoagulant, antivenom, antiprotozoal, antifungal, antibacterial,

antidiabetic, antiulcer, antimutagenic, anticarcinogenic, antiviral, and antioxidant [1]. Curcumin has been expected certain barriers to use such as low absorption rates and therefore, many processing methods have been proposed to increase its bioavailability [2–5]. Theracurmin (CR-033P) is a form of curcumin that is known to be more bioavailable [6] and is characterized by submicron particle size.

According to a clinical study, CR-033P exhibited a remarkable increase in both maximum blood concentration (C max) and the area under the concentration–time curve (AUC), reaching levels 20 and 42 times higher, respectively, than those observed with conventional curcumin [5]. A recently developed product, Theracurmin Super (TS-P1), represents a highly absorbable form of curcumin. Studies have demonstrated that its administration leads to a blood concentration of curcumin twice as high as that achieved with CR-033P [7]. Moreover, we conducted an exploration into the impacts of TS-P1 and CR-033P on symptoms associated with the common cold and immune function. TS-P1 exhibited a blood concentration of total curcumin twice as high as that observed with CR-033P, and it also demonstrated a greater potential for enhancing immune function compared to CR-033P [8]. The disparity between TS-P1 and CR-033P lies in the unique quality of CR-033P, which boasts high dispersibility. This characteristic is attained through the micronization of ordinary crystalline curcumin into submicron particles using gum ghatti [6]. In contrast, TS-P1 is synthesized by subjecting ordinary crystalline curcumin to a process of melting, resulting in the formation of amorphous curcumin, a non-crystalline substance characterized by elevated solubility and/or an increased dissolution rate [7]. If the bioavailability is high, it is expected to have a strong health effect on humans. In fact, curcumin is known to inhibit some metabolic enzymes in the liver, and in some cases liver damage has been reported [9].

So far, safety has been confirmed in CR-033P ingestion of 180 mg/day as curcumin content for 12 weeks and 900 mg/day as curcumin content for 4 weeks [10]. The safety of CR-033P or TS-P1 ingestion of 150 mg/day as curcumin content for 12 weeks and TS-P1 ingestion of 750 mg/day as curcumin content for 4 weeks has not yet been confirmed.

Therefore, we conducted a study to investigate the safety of a 12-week consistent intake of CR-033P or TS-P1 (150 mg/day as curcumin content) to healthy Japanese adults as potential marketers of foods containing CR-033P or TS-P1. Additionally, we conducted a study to assess the safety of 4-week continuous intake of TS-P1 at a higher level (750 mg/day as curcumin content).

METHODS

Study design: Both Trial 1 and Trial 2 were structured as randomized, placebo-controlled, double-blind, parallel-group investigations. The protocols of these studies obtained approval from the ethics committee at the Takara Clinic, Medical Corporation Seishinkai, Tokyo, Japan (Trial 1: Approval was granted on November 22, 2021, with the Approval Number 2111-02164-0043-1C-TC; Trial 2: Approval was granted on August 24, 2022, with Approval N umber 2208-02164-0046-02-TC). The research experiments were conducted in strict adherence to the principles set forth in the Declaration of Helsinki (2013), the ethical guidelines governing medical and health research involving human participants in Japan, and the broader framework of medical ethics.

Subjects:

In Trial 1 and Trial 2, the inclusion criteria encompassed healthy Japanese individuals aged 20 and above, deemed eligible for participation by the principal investigator.

The exclusion criteria for both Trial 1 and Trial 2 were delineated as follows:

1. Individuals with a history of or currently undergoing medical treatment for conditions such as malignant tumors, heart failure, myocardial infarction, arrhythmia, liver disorders, kidney disorders, cerebrovascular disorders, rheumatism, diabetes mellitus, dyslipidemia, hypertension, and any other chronic diseases.
2. Individuals with a pacemaker or an implantable cardioverter defibrillator (ICD) were also excluded from participation.
3. Also excluded were individuals currently consuming "Foods for Specified Health Uses," "Foods with Functional Claims," or medications (including herbal medicines) and supplements daily.
4. Individuals with allergies to medicines and/or products related to the test food were considered ineligible for participation.
5. Additionally, individuals who were pregnant, lactating, or planning to become pregnant during these trials were excluded.
6. Subjects currently experiencing symptoms of COVID-19 were also excluded from participation.

Participants who have entered into participation agreements for additional clinical trials within the 28-day period preceding their consent for involvement in this trial or anticipate engaging in another trial concurrent with these proceedings were excluded. Detailed eligibility criteria for Trial 1 and Trial 2 can be found in UMIN000046160 (Trial 1) and UMIN000048797 (Trial 2), both filed with the UMIN Clinical Trials Registry (UMIN-CTR).

All participants were registered on the website (https://www.go106.jp) managed by ORTHOMEDICO Inc., Tokyo, Japan. The study protocols were thoroughly explained to all participants, and they signed the informed consent document at the ORTHOMEDICO Inc. office before participating in the study. No members of sponsoring or funding companies participated in the studies. Seishinkai, Takara Clinic in Tokyo, Japan, oversaw data evaluation, subject health management, and conducted examinations, collaborating with Nerima Medical Association, Minami-machi Clinic for the study.

Intervention: As test foods, foods containing CR-033P and excipients (CR-033P group), foods containing TS-P1 and excipients (TS-P1 group), or placebos in which excipients were substituted for ingredients other than those involved Food (placebo group; P group) was prepared by Theravalues Corporation.

The test foods, all presented in capsule form, underwent verification during the ethics review before the study commencement. The ethics committee members confirmed that the test foods and capsules were deemed identical in color, odor, and flavor.

Additionally, in both Trial 1 and Trial 2, all the test foods were in capsule form, and it was ascertained during the ethics review before the study that the test foods and the placebo foods could not be distinguished by color, odor, or flavor. The intervention conditions for Trial 1 and Trial 2 were as follows, respectively. The intervention conditions for Trial 1 and Trial 2 were defined as follows:

Trial 1: The participants were asked to take 4 total capsules of either CR-033P, TS-P1, or placebo twice per day with water after breakfast and dinner (2 capsules each meal) for 8 weeks. Both CR-033P and TS-P1 were designed to be ingested at 150 mg/day as curcumin.

Trial 2: The participants were asked to take 20 total capsules of either TS-P1 or placebo twice per day with water after breakfast and dinner (10 capsules each meal) for 4 weeks. TS-P1 was designed to be ingested at 750 mg/day as curcumin.

Safety Evaluation items: The safety evaluation time points of Trial 1 and Trial 2 were as follows:

Trial 1: Safety was evaluated at screening (Scr) and at 8 (8wks) and 12 weeks (12wks) after the test compound intake.

Trial 2: Safety was evaluated at screening (Scr) and at 2 (2wks) and 4 weeks (4wks) after the test compound intake.

Evaluation items were physical examination, urinalysis, and blood tests, and the following contents were carried out respectively.

Anthropometric and physical evaluations covered measurements of weight, body mass index (BMI), body fat percentage, and body temperature, as well as recordings of systolic blood pressure, diastolic blood pressure, and pulse rate. Height was measured at the briefing session and used to calculate BMI. The pulse rate was measured only in Trial 1.

During urinalysis, urine samples were gathered to assess protein, glucose, urobilinogen, bilirubin, ketone bodies, pH, and occult blood levels. These collected samples were handed over to LSI Medience Corporation (Chiyoda, Tokyo, Japan), and each parameter was evaluated in adherence to global standards. Urobilinogen, bilirubin, and ketone bodies were measured only in Trial 1. Hematological assessments covered the following parameters: platelet count, mean corpuscular volume (MCV), percentage of neutrophils, erythrocyte count, hemoglobin, differentiation of white blood cells (percentage of lymphocytes, monocytes, eosinophils, and basophils), leukocyte count, mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). These evaluations were conducted to comprehensively analyze the hematological profile of the subjects. Biochemical assessments covered a wide range of parameters: total bilirubin, direct bilirubin, indirect bilirubin, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, alkaline phosphatase, lactate dehydrogenase, leucine aminopeptidase, cholinesterase, urea nitrogen, creatinine, uric acid, creatine kinase, calcium, serum amylase, total protein, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, glycoalbumin, serum iron, sodium, potassium, chloride, inorganic phosphorus, glucose, hemoglobin A1c (NGSP), and nonspecific immunoglobulin (IgE). These examinations aimed to comprehensively analyze the biochemical profile of the subjects. The measurement of each parameter was conducted by LSI Medience Corporation following the global standard. Also, of these MCV, MCH, MCHC, alkaline phosphatase, lactate dehydrogenase, leucine aminopeptidase, direct bilirubin, indirect bilirubin, cholinesterase, creatine kinase, calcium, glycoalbumin, serum iron, inorganic phosphorus and nonspecific IgE was measured only in Trial 1.

All participants recorded their intake of the test food and any change in their physical condition in a diary. Participants recorded any side effects or adverse events in a diary and reported them once they became aware of them. Side effects were collected only in Trial 1. If a symptom recognized as an adverse event occurs, the principal investigator will promptly take necessary and appropriate measures. They will also assess whether the study should continue and decide whether to unlock the emergency key. Furthermore, the principal physician assessed the relationship between the adverse events and the test food and documented the findings in writing. When taking any prescription drug or over the counter (OTC) drug (drugs requiring guidance or over-the-counter drugs), inform the contracting institution of the fact that they are taking the drug, the type of drug used, the date of use, the amount used, the reason for use, etc. reported. In addition, to confirm the health status of the

test participants, we conducted interviews and diet surveys using the nutritional value diary CAND (Calorie and Nutrition Diary) [11] on each test day. Study participants were excluded from these studies if they withdrew consent or had protocol deviations.

Sample size:

Trial 1: No prior study has investigated the impact of consuming test foods continuously for 12 weeks on the persistence of common cold symptoms in healthy Japanese adults. Hence, our foresight indicates a notable variance in the aggregate duration of common cold symptoms between the TS-P1 or CR-033P cohort and the P group, employing an effect size (d) of 0.80, as proposed by Cohen [12]. The determination of sample size involved considerations of an assumed effect size (d) of 0.80, a significance level (α) set at 0.05, and a statistical power (1−β) of 0.80. As a result, a sample size of 78 was established, with 26 participants allocated to each group. To enhance statistical power within the budget, the sample size was set at 90 subjects (30 per group), and the recalculated statistical power (1−β) was 86.1. Accounting for potential dropouts or subjects deviating from the protocol at 10% during this trial, the total number of subjects included was set at 99 (33 participants per group).

Trial 2: The number of participants detected with 95% accuracy at least one or more adverse events with an incident rate of 15% in each group was determined from the following equation (1).

$$\boldsymbol{n = log(1-p) \,/\, log\,(1-r)}$$
$$n\text{: The number of participants,}$$
$$r\text{: The adverse event rate, } p\text{: The detection rate} \quad (1)$$

Based on equation (1), the calculated sample size per group was determined to be 20 participants. To account for potential dropouts, two additional participants were included in each group, resulting in a total of 22 participants per group. Therefore, we decided to enroll 44 participants in the study.

Selection, randomization, and blinding: In Trial 1, the principal investigator enrolled 99 eligible participants out of a total of 143 who submitted informed consent, and in Trial 2, the principal investigator enrolled 44 eligible participants out of a total of 73 who submitted informed consent. Test compounds were provided by Theravalues Corporation to the contract research organization (CRO). After declaring indistinguishability between the test compounds, a person in charge of shipping from the CRO gave the test compound code to an allocation controller who was not directly involved in the trials. An allocation controller allocated all patients on the same day after the number of enrolled cases reached the target number of cases set in the protocol. An allocation controller generated random numbers on a computer and allocation adjustment of (i) sex, (ii) age, and (iii) immunity score for Trial 1 and (i) sex and (ii) age for Trial 2. A randomization table was created by the complete randomization method with factors, and the subjects were equally but randomly assigned to each group. Test foods were distributed to each subject based on the coded allocation table, which was only accessible to the person in charge of shipping. After the test foods were dispatched, the allocation table was securely stored until the key opened. The group assignments remained undisclosed to the sponsor, chief medical practitioner, secondary healthcare provider, shipping logistics overseer, and the comprehensive CRO unit (encompassing the trial overseeing head, trial implementation supervisor, monitoring liaison, statistical analysis overseer, and supporting personnel). Similarly, individuals from medical facilities, ethics committee associates, contracted laboratory representatives, and other affiliated parties engaged in these investigations were intentionally kept unaware of the assigned groups.

The allocation controller locked the allocation table until the number of cases, subjects, and analysis methods were finalized.

Statistical analysis: The two-sided statistical analyses was established at a significance level of 5%. Data analysis was carried out using Windows SPSS version 23.0 (IBM Japan, Ltd., Tokyo, Japan). Moreover, an analysis with a primary outcome focus was performed. Nevertheless, the challenge of multiplicity pertaining to diverse variables and various temporal junctures in secondary outcomes was not considered within the scope of this investigation. The participants' background was collected demographically and showed the participants' gender, age, height, systolic and diastolic blood pressure, and pulse rate. Since pulse rate was an item measured only in Trial 1, it was not included in the background of participants in Trial 2.

For side effects and adverse events, we calculated the number of cases and incidence rate for each group, determined the difference in the incidence rate, and established its 95% confidence interval. Comparisons between groups were conducted using the chi-square test.

Reported were the ascertained metrics for primary and secondary results, encompassing averages, standard deviations (SD), distinctions between groups, and standard errors (SE). Group differences were presented alongside their 95% confidence interval. A linear model with the group as a factor was performed to determine a group comparison between the TS-P1 or CR-033P group and the P group.

Urinalysis and peripheral blood test values were within the standard values for Scr. Number of applicable cases, applicable rate, and applicable rate for each group in cases where each measured value of urinalysis and peripheral blood test values changed outside the standard values after intervention The difference and its 95% confidence interval were calculated, and comparison between groups was performed by the chi-square test.

Urinalysis and hematological test values were within the standard values for Scr. number of applicable cases, applicable rate, and applicable rate for each group in cases where each measured value of urinalysis and peripheral blood test values changed outside the standard values after intervention the difference and its 95% confidence interval were calculated, and comparison between groups was performed using the chi-square test. The difference in the incidence rate and the difference in the applicable rate were calculated by subtracting the value in the P group from the value in the TS-P1 group or CR-033P group. Additionally, the primary examiner or auxiliary assessor carried out individualized safety evaluations, affirming the absence of medically concerning alterations upon sustained consumption of the experimental food.

RESULTS

Analysis set:

Trial 1: Illustrated in Figure 1 is the schematic representation of participant progression. Recruitment took place between November 25, 2021, and January 14, 2022, with the testing phase spanning from November 25, 2021, to May 31, 2022. While each subject received the treatment prescribed for them, four participants (one in the TS-P1 group and three in the P group) never showed up for their examination at 8 weeks. Excluding four cases where participants did not receive intervention after allocation, the safety analysis population (SAF) consisted of 95 participants in the final dataset, distributed across groups as follows: 32 participants in the TS-P1 group, 33 participants in the CR-033P group, and 30 participants in the P group. Table 1 shows the intention to treat (ITT) and the background of participants in the SAF. There was no significant difference in the participants' backgrounds.

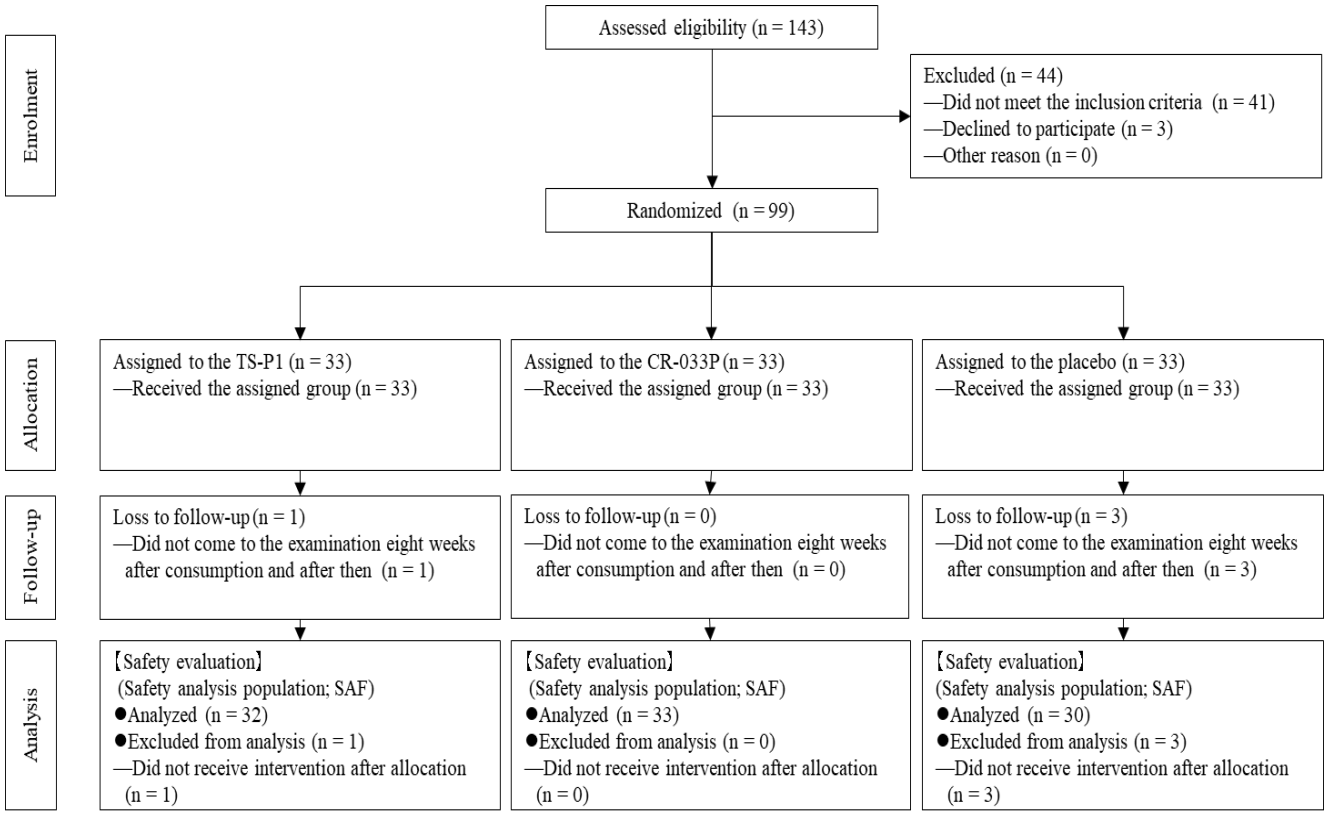


Figure 1. The flowchart of participation in Trial 1

Table 1. Subjects` background information in Trial 1

		ITT			SAF		
		TS-P1group (n = 33)	**CR-033P group (n = 33)**	**P group (n = 33)**	**TS-P1group (n = 32)**	**CR-033P group (n = 33)**	**P group (n = 30)**
Sex	Men	13 (39.4%)	14 (42.4%)	13 (39.4%)	13 (40.6%)	14 (42.4%)	11 (36.7%)
	Women	20 (60.6%)	19 (57.6%)	20 (60.6%)	19 (59.4%)	19 (57.6%)	19 (63.3%)
Age (years)	Mean (SD)	49.0 (11.8)	49.0 (10.9)	49.2 (11.7)	48.4 (11.4)	49.0 (10.9)	50.4 (11.4)
	Min-Max	27–75	28–74	25–70	27–75	28–74	25–70
Height (cm)	Mean (SD)	162.6 (7.8)	163.9 (8.1)	163.4 (9.7)	162.8 (7.8)	163.9 (8.1)	162.7 (9.5)
	Min-Max	153.0–179.0	148.0–178.0	147.0–183.0	153.0–179.0	148.0–178.0	147.0–179.5
Body weight (kg)	Mean (SD)	58.1 (13.4)	57.8 (10.6)	62.4 (14.8)	58.6 (13.3)	57.8 (10.6)	60.4 (12.8)
	Min-Max	39.3–105.6	40.5–79.4	41.8–103.0	39.3–105.6	40.5–79.4	41.8–92.2
BMI (kg/m^2)	Mean (SD)	21.9 (4.6)	21.4 (2.7)	23.2 (4.2)	22.0 (4.6)	21.4 (2.7)	22.7 (3.8)
	Min-Max	16.8–42.8	16.6–27.8	16.3–32.9	16.8–42.8	16.6–27.8	16.3–32.9
Systolic blood pressure(mmHg)	Mean (SD)	116.0 (13.4)	116.0 (15.0)	120.9 (17.4)	114.8 (11.6)	116.0 (15.0)	121.5 (18.1)
	Min-Max	93–155	89–144	92–156	93–143	89–144	92–156
Diastolic blood pressure(mmHg)	Mean (SD)	74.7 (8.7)	76.0 (11.7)	77.1 (11.3)	74.3 (8.6)	76.0 (11.7)	77.2 (11.9)
	Min-Max	57–92	58–97	58–99	57–92	58–97	58–99
Pulse rate (bpm)	Mean (SD)	74.7 (10.8)	73.2 (10.5)	75.7 (10.2)	74.8 (11.0)	73.2 (10.5)	75.8 (10.5)
	Min-Max	55–101	55–94	63–104	55–101	55–94	63–104

Sex distribution is presented as the count and percentage of cases within each group. In other items were expressed as the mean and standard deviation, Min, Max. ITT, intention to treat; SAF, safety analysis population.

Trial 2: Figure 2 illustrates the participant flowchart. Recruitment took place between August 25, 2022, and September 29, 2022, while the testing period extended from August 25, 2022, to November 27, 2022. Although each subject received their prescribed treatment, two participants (both from the P group) missed the 2-week examination, and one participant (from the TS-P1 group) missed the 4-week examination. The number of cases of SAF was 42 participants in the final (22 participants in the TS-P1 group, and 20 participants in the P group) excluding 2 cases of did not receive the intervention after allocation. Table 2 shows the ITT and the background of participants in the SAF. There was no significant difference in the participants' backgrounds.

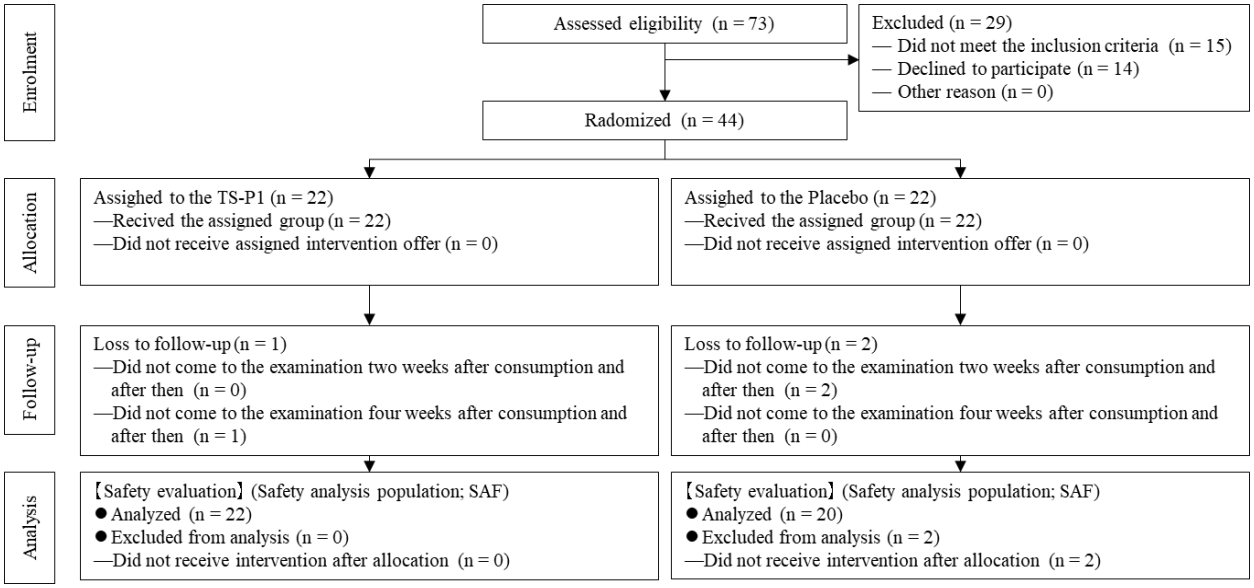


Figure 2. The flowchart of participation in Trial 2

Table 2. Subjects` background information in Trial 2

		ITT		SAF	
		TS-P1 group (n = 22)	**P group (n = 22)**	**TS-P1 group (n = 22)**	**P group (n = 20)**
Sex	Men	7 (31.8%)	8 (36.4%)	7 (31.8%)	8 (40.0%)
	Women	15 (68.2%)	14 (63.6%)	15 (68.2%)	12 (60.0%)
Age (years)	Mean (SD)	42.3 (11.3)	42.4 (11.8)	42.3 (11.3)	42.3 (12.3)
	Min-Max	21-62	19-66	21-62	19-66
Height (cm)	Mean (SD)	163.2 (8.2)	161.7 (7.8)	163.2 (8.2)	162.0 (7.7)
	Min-Max	153.1-184.8	148.6-178.7	153.1-184.8	148.6-178.7
Body weight (kg)	Mean (SD)	57.6 (5.7)	58.0 (13.4)	57.6 (5.7)	58.1 (13.6)
	Min-Max	45.7-67.1	45.0-90.4	45.7-67.1	45.0-90.4
BMI (kg/m^2)	Mean (SD)	21.7 (2.0)	22.0 (3.8)	21.7 (2.0)	22.0 (4.0)
	Min-Max	18.2-25.3	16.1-30.2	18.2-25.3	16.1-30.2
Systolic blood pressure (mmHg)	Mean (SD)	113.0 (15.5)	117.0 (13.1)	113.0 (15.5)	116.3 (13.5)
	Min-Max	92-151	91-142	92-151	91-142
Diastolic blood pressure (mmHg)	Mean (SD)	71.2 (12.4)	78.2 (12.4)	71.2 (12.4)	77.7 (12.8)
	Min-Max	53-101	51-108	53-101	51-108

Values of sex are shown as number of cases and percentage of cases in each group. In other items were expressed as the mean and standard deviation, Min, Max. ITT, intention to treat; SAF, safety analysis population.

Safety evaluation [Trial 1]: Throughout the study period, no side effects were identified under the given study conditions. However, four adverse events were confirmed in the TS-P1 group (ID65710 dry eye, ID65821 and ID65838 common cold symptoms, ID66761 allergic rhinitis), and the incidence in the TS-P1group was 12.5%. Meanwhile, 7 cases of adverse events were confirmed in the CR-033P group (ID65720 allergic rhinitis, swelling, constipation, ID65994 allergic rhinitis, abdominal pain, ID66311 common cold symptoms, ID66342 allergic rhinitis, ID66530 muscle tear, ID66537 cough, high blood pressure, ID66640 allergic rhinitis, throat discomfort, throat pain, blocked nose, itching of eyes), and the incidence in the CR-033P group was 21.2%.

On the other hand, adverse events were confirmed in 6 cases of adverse events in P group (ID65822 chills, ID66005 stomachache, ID66288 toothache, ID66660 allergic rhinitis, dysmenorrhea, throat pain, headache, ID66799 common cold symptoms, sugar absorption suppression, ID66804 diarrhea, loose stools, abdominal pain, abdominal distension), and the incidence in the P group was 20.0% (Table 3). However, the adverse events observed in the test food (TS-P1 and CR-033P) group and the P group were resolved using the drug and were determined by the principal physician to not be due to the test food intake. In addition, we checked the percentage of cases in which each measured value of urinalysis and peripheral blood test, which was within the standard value for Scr, changed outside the standard value after the intervention (Appendix 1), and each item was confirmed on an individual basis. Given this, there were no observed medically problematic changes associated with the ongoing consumption of the test food.

Table 3. Side effects and adverse event incidence in Trial 1.

Item		n	Number of cases of expression	Expression (%)	Comparison between groups (vs. P group)		
					⊿ (%)	95%CI	P
Side effect incidence	TS-P1group	32	0	0.0	0.0	NA	NA
	CR-033P group	33	0	0.0	0.0	NA	NA
	P group	30	0	0.0	-	-	-
Incidence of adverse events	TS-P1group	32	4	12.5	−7.5	(−25.8, 10.8)	0.502
	CR-033P group	33	7	21.2	1.2	(−18.8, 21.2)	1.000
	P group	30	6	20.0	-	-	-

NA, not available, ⊿: Group difference, 95%CI: 95% Confidence interval / P: Significance probability

Safety evaluation [Trial 2]: Concerning the incidence of adverse events, the primary outcome of this study, four adverse events were confirmed in the TS-P1 group during the study period. These events included ID80933 (urticaria), ID80935 (insomnia), ID82407 (common cold symptoms), and ID82415 (eczema, arthralgia). The incidence in the TS-P1 group was 18.2%. On the other hand, in the P group, 3 adverse events were confirmed (ID81043 headache, ID81333 allergic rhinitis, ID81459 allergic rhinitis), and the incidence in the group was 15.0% (Table 4).

However, the adverse events observed in the TS-P1 group, and the P group were resolved by the use of the drug and were determined by the principal physician to

not be due to the test food.

Furthermore, we assessed the percentage of cases in which each measured value of urinalysis and peripheral blood test, initially within the standard value for Scr, deviated outside the standard value after the intervention (refer to Appendix 2). Each item was individually examined, and it is noteworthy that no medically problematic changes were observed with the sustained consumption of the test food.

Table 4. Adverse event incidence in Trial 2.

Item	**TS-P1group**				**Comparison between groups (vs. P group)**		
		n	Number of cases of expression	Expression (%)	⊿ (%)	95%CI	P
Incidence of adverse events	TS-P1group	22	4	18.2	3.2	(−19.4, 25.7)	1.000
	P group	20	3	15.0	-	-	-

⊿: Group difference, 95%CI: 95% Confidence interval / P: Significance probability

DISCUSSION

Japan is one of the countries where the proportion of elderly people has increased more than any other country and according to the results of the Population Census of the Statistics Bureau of Japan, the proportion of the total population aged 65 and over is 29.1% and is expected to continue to rise. [13]. In anticipation of an increasing proportion of elderly individuals in the population, the Ministry of Education, Science, Sports, and Culture (MHLW) initiated a research and development project on food functionality in 1984. Subsequently, in April 2001, a new regulatory system known as "Foods with Health Claims (FHC)" was implemented [14]. In Japan, functional substance and health foods that have been clinically evidence-proven are classified as Foods for Specific Health Uses (FOSHU) and Foods with functional claims and are clearly distinguished from their status as ``foods'' or ``so-called health foods``.

FHC was introduced in April 2001 to offer scientifically supported products to consumers. This system ensures the safety and effectiveness of foods with functional claims by requiring sellers to submit dossiers to the Consumer Affairs Agency (CAA). Not only data showing effectiveness but also data showing the safety levels that are required, and once a series of documents have been submitted to the CAA, the product is marketed [15]. There is still no consensus on the definition of functional foods (FF), and according to many organizations, the medical community lacks a comprehensive process for its classification. For this reason, the Functional Food Center (FFC) has advocated for a multi-step process for developing functional food products and bringing them to market without the necessity of categorizing them as established items [16]. In the classification proposed by FFC, Foods with functional claims corresponds to Category C functional food product.

The safety of foods with functional claims products' is typically assessed using information about the food experience. If the information on food experience is not sufficient for safety, a safety test will be conducted to evaluate safety. Regarding food in capsule form, by treating it as processed food that is counted as a supplement, it is considered that there is a possibility of excessive intake considering the recommended daily intake amount, and safety aspects of excessive intake are also required.[17]. Against this background, studies that

evaluate not only scientific effectiveness but also safety are considered important in Japan, and many studies have been conducted and published [18 -20]. Clinical trials for safety evaluation in Japan have established study design, and the long-term safety of foods is assessed for 12 weeks, and excessive intake is assessed by consuming 3 times or more of the recommended intake amount for 4 weeks [21]. However, there are no rules regarding these tests other than the period and intake. Looking at previous studies, there are open label trials, one-arm trials, and various trial designs have been conducted [18-20].

Curcumin accounts for 75% of total curcuminoids, has been ingested for a long time, and was considered safe having been listed by the US Food and Drug Administration (USFDA) as "Generally Recognized as Safe" (GRAS). In addition, preparations with increased curcumin absorption bioavailability have been developed and are attracting high levels of attention as supplements for maintaining health [22, 23]. On the other hand, there have been case reports of hepatotoxicity induced by curcumin supplement intake, raising concerns among consumers regarding the use of curcumin supplements [24-27]. Nevertheless, despite the lack of conclusive proof of toxicity, reservations have been voiced regarding the inclusion of enhancers impeding the body's vital detoxification pathways, potential contamination of synthetic curcumin, the existence of heavy metals, chromates, illicit dyes, non-steroidal anti-inflammatory drugs, and various other impurities [24-27]. Against this background, some recent studies have been published on safety tests using curcumin supplements [10,28]. These studies were the only other reports on curcumin overdose study [10].

Against this background, we conducted a safety evaluation of excessive and long-term intake of curcumin using a double-blind placebo-compared design.

This study conducted two trials and investigated the safety of 4-week excessive and 12- week long term intake of TS-P1 and 12- week long term intake of CR-033P on healthy Japanese adults.

In the long-term intake study, participants were given TS-P1-containing food, CR-033P-containing food (150 mg/day of curcumin), or placebo as the test food. The study was conducted under the condition of taking 2 tablets before breakfast and 2 tablets before dinner with water 10 to 15 minutes before meals.

On the other hand, in the excessive intake study, participants were given either a TS-P1-containing food (750 mg/day of curcumin) or a placebo. The study was conducted under the condition of taking 2 tablets before breakfast and 2 tablets before dinner with water 10 to 15 minutes before meals.

As a result of the study, adverse events were confirmed in some participants in Trial 1 and Trial 2, but none of the symptoms were caused by curcumin and were considered to be changes in physical condition caused by other factors than the test food. In addition, turmeric, the raw material for TS-P1 and CR-033P, is known to inhibit some of the metabolic enzymes in the liver, and some health hazards due to liver dysfunction have been reported [9]. After confirming the liver function marker data, there was no significant difference between the P group in either Trial 1 or Trial 2, and it was judged that the changes in liver function markers after the intervention were not caused by continuous intake of the test food. In addition, the other hematological tests were confirmed, and no medically problematic changes due to ingestion of the test food.

In a previous study, CK was significantly different from the placebo group, but the change was within the reference range [10]. In addition, Pancholi et al. showed changes in biochemical parameters over 90 days from baseline to the end of the study [28]. And in a previous results showed that alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT), Fasting Blood Sugar, Total Cholesterol (TC), LDL cholesterol and MCH were significantly reduced compared to baseline. And MCH

was significantly increased compared to baseline. However, those numbers were changes in the reference range [28]. In a previous study of 8-week intake, there was a significant difference in total bilirubin, Ibil, and triglyceride from placebo group, but the changes were showed to be within the reference range [9]. Based on the results of such previous studies, we further confirmed whether there were any items outside the reference range (Appendix 1, 2). As a result, the item that showed outside the standard value during the test period was the uric acid level of TS-P1 8 weeks after administration, as shown in Appendix 1.

The uric acid levels were examined in each subject case and confirmed that no medically problematic changes occurred due to the intake of the test food.

Drawing conclusions from Trial 1 and Trial 2 outcomes, it is substantiated that the safety of ingesting 150 mg/day curcumin equivalent of CR-033P or TS-P1 over a 12-week duration, alongside the ingestion of 750 mg/day curcumin equivalent of TS-P1 for 4 weeks, has been confirmed.

CONCLUSION:

We investigated the safety of high bioavailability curcumin TS-P1 and CR-033P in healthy Japanese adults. These trials found that the safety of consuming 150 mg/day of curcumin is equivalent to TS-P1 or CR-033P for 12 weeks and 750 mg/day of curcumin equivalent of TS-P1 for 4 weeks. Therefore, the intake of TS-P1 and CR-033P to receive possible health benefits proves to be safe in healthy individuals.

List of abbreviations: CRO, contract research organization; ITT, intention to treat; SAF, safety analysis set; MHLW, Ministry of Education Science Sports and Culture; FHC, Foods with Health Claims; FOSHU, Foods for Specific Health Uses; FF, Functional Food; CAA, Consumer Affairs Agency; FFC, Functional Food Center; USFDA, US Food and Drug Administration; GRAS, Generally Recognized as Safe; mean corpuscular hemoglobin, MCH.

Competing interests: The sponsor of this study, Theravalues Corporation, entrusted ORTHOMEDICO Inc. with conducting the study. Hyunjin Lee is a member of Theravalues Corporation, and Naoko Suzuki is employees of ORTHOMEDICO Inc. Tsuyoshi Takara (MD), the principal investigator of this study, is a director of Medical Corporation Seishinkai, Takara Clinic, and he monitored all of the conditions of the participants.

Authors' contributions: Conceptualization, Hyunjin Lee, Yoshitaka Kuwabara, Akiko Hirose, Toshihiro Kakinuma, Asami Baba Tsuyoshi Takara; Data curation, Toshihiro Kakinuma; Formal analysis, Toshihiro Kakinuma; Funding acquisition, Hyunjin Lee, Yoshitaka Kuwabara, Akiko Hirose; Investigation, Asami Baba, Tsuyoshi Takara; Methodology, Toshihiro Kakinuma, Asami Baba; Project administration, Hyunjin Lee, Yoshitaka Kuwabara, Akiko Hirose, Asami Baba, Tsuyoshi Takara; Resources, Hyunjin Lee, Yoshitaka Kuwabara, Akiko Hirose, Asami Baba, Toshihiro Kakinuma; Supervision, Tsuyoshi Takara; Software, Toshihiro Kakinuma; Supervision, Tsuyoshi Takara; Validation, Asami Baba; Visualization, Asami Baba; writing—original draft preparation, Hyunjin Lee, Yoshitaka Kuwabara, Akiko Hirose; Writing – review & editing, Asami Baba, Tsuyoshi Takara.

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