การศึกษาแบบเพิ่มขนาดรับประทานเพื่อกำหนดปริมาณที่ปลอดภัยของน้ำจากเนื้อผลกาแฟเข้มข้นในคนสุขภาพดี

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บทคัดย่อ

ในอดีตส่วนเนื้อและเปลือกนอกของกาแฟ (coffee cherry pulp) ถือเป็นขยะที่ได้จากอุตสาหกรรมการผลิตกาแฟ ปัจจุบันได้รับความสนใจนำมาเป็นอาหารใหม่ที่อาจมีประโยชน์ต่อสุขภาพ อย่างไรก็ตามยังไม่ทราบปริมาณสูงสุดที่รับประทานได้อย่างปลอดภัยของอาหารใหม่นี้ การศึกษาแบบเพิ่มขนาดรับประทานนี้มีวัตถุประสงค์เพื่อประเมินความปลอดภัยและความทนและกำหนดปริมาณที่ปลอดภัยของน้ำจากเนื้อผลกาแฟเข้มข้น ในช่วงที่ 1 เป็นการศึกษาแบบเพิ่มขนาดสำหรับรับประทานครั้งเดียว อาสาสมัคร 6 คน ได้รับน้ำจากเนื้อผลกาแฟเข้มข้น (ความเข้มข้น 50 °Brix) จากขนาดน้อยไปมากเป็นขั้นๆคือ จาก 1 ถึง 14 กรัม วันละ 1 ครั้ง แต่ละขั้นห่างกัน 1 วัน รวมเวลา 14 วัน ในช่วงที่ 2 เป็นการศึกษาแบบเพิ่มขนาดสำหรับรับประทานหลายครั้งต่อเนื่อง อาสาสมัคร 12 คน ได้รับน้ำจากเนื้อผลกาแฟเข้มข้น จากขนาดน้อยไปมากเป็นขั้นๆคือ จาก 1 ถึง 14 กรัม วันละ 2 ครั้ง (รวมเป็น 2 - 28 กรัมต่อวัน) แต่ละขั้นให้รับประทานเป็นเวลา 2 สัปดาห์ การวัดผลความปลอดภัยและความทนวัดจากอาการไม่พึงประสงค์ประเมินด้วยสมุดบันทึกของอาสาสมัคร และค่าเคมีคลินิกในเลือด ผลการศึกษาพบว่า ไม่มีอาสาสมัครเกิดอาการไม่พึงประสงค์ใดๆ เมื่อรับประทานเครื่องดื่มดังกล่าวครั้งเดียวหรือหลายครั้งต่อเนื่องที่ระดับสูงสุด ค่าเคมีคลินิกเฉลี่ยอยู่ในระดับปกติตลอดการศึกษา ดังนั้นปริมาณสูงสุดที่รับประทานได้อย่างปลอดภัยของน้ำจากเนื้อผลกาแฟเข้มข้นคือ 14 กรัม วันละ 2 ครั้ง ซึ่งเท่ากับ 28 กรัมต่อวัน (ของแข็งทั้งหมด 14 กรัม) ในอนาคตควรมีการศึกษาความปลอดภัยในระยะยาวของน้ำจากเนื้อผลกาแฟเข้มข้นต่อไป

คำสำคัญ: น้ำจากเนื้อผลกาแฟเข้มข้น การศึกษาแบบเพิ่มขนาดรับประทาน ความปลอดภัย อาสาสมัครสุขภาพดี การทดลองทางคลินิก ขนาดรับประทานสูงสุดที่ทนได้

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A Dose-Escalation Study for Identifying Safe Doses of Coffee Cherry Pulp Juice Concentrate in Healthy Subjects

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Abstract

Previously viewed as industrial waste in coffee manufacture, coffee cherry pulp now receives attention as a functional food with potential health benefits. However, the maximum safe dose of this novel ingredient for human consumption was unknown. This dose-escalation study aimed to evaluate the safety and tolerability of coffee cherry pulp juice concentrate in healthy subjects and identify the maximum tolerated dose (MTD). In the single ascending dose phase, six participants received a single dose daily in a stepwise escalation from 1 to 14 g of coffee cherry pulp juice concentrate (50 °Brix), within 14 days. In the multiple ascending dose phase, twelve subjects received a stepwise escalating dose from 1 to 14 g of coffee cherry pulp juice concentrate twice daily (2-28 g per day). Each dose has been repeated for two weeks. Safety and tolerability were assessed by subject diaries and clinical laboratory tests. The results show no adverse events occurred after single or repeated doses. The average blood biochemical values are all in the normal range throughout the study. The maximum tolerated dose of coffee cherry pulp juice concentrate in short term is 14 g twice daily, which equals 28 g per day (total solid of 14 g). Future studies for the long-term safety of coffee cherry pulp juice concentrate are warranted.

Keywords: Coffee cherry pulp juice concentrate, Dose-escalation study, Safety, Healthy subjects, Clinical trial, Maximum tolerated dose

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Introduction

Coffee is one of the most popular beverages and traded agricultural commodities worldwide. The common coffee drink is made from roasted coffee beans, which is the seed inside coffee cherry fruit. The coffee bean is produced either by dry processing, or by wet processing, which is a multi- step procedure including, harvesting, drying, pulping, fermentation, drying, hulling, and cleaning, then roasting and grinding [1-5]. These processes generated a large amount of waste and by- products including coffee cherry husks, coffee pulp, coffee silverskin, and spent grounds [6- 7]. Coffee pulp is the first by- product obtained during the wet processing and semi- dry processing of coffee cherries, representing 40- 50 % of the coffee berry' s wet weight (about 29 % dry-weight) [8-9]. The production of coffee pulp residue globally is estimated at 9.4 million tons per year, which can cause environmental pollution such as lowering oxygen in the river, reducing soil flora diversity, and releasing methane and nitrous oxide from the fermentation of coffee pulp [9]. Thus, the utilization of coffee pulp is a global need for proper industrial waste management.

Recently, coffee pulp receives attention for possible health benefits owing to its bioactive compounds such as polyphenols, caffeine, tannins proantho- cyanidins, anthocyanidins, and caffeoyl- quinic acid and its derivative [8- 11]. Previous studies found that coffee pulp aqueous extract (CPE) rich in chlorogenic acid (CGA), caffeine, epicatechin (EC), and catechin, have a cholesterol-lowering effect both *in vitro* and *in vivo* models via inhibition of intestinal cholesterol absorption [12]. Moreover, the combination of CPE with simvastatin reduced hyperlipidemia, insulin resistance, and hepatic steatosis in high- fat diet- induced obese rats [13]. CPE also reduced oxidative stress and hepatic lipid accumulation in hepatocellular carcinoma (HepG2) [14].

Normally, coffee pulp refers to the skin (exocarp) and pulp (mesocarp). The skin is thick and bitter, while the pulp is intensely sweet with a grape- like texture [15]. The coffee pulp can be processed into various food commodities like jam, juice, concentrate, jelly, flavoring, and alcoholic beverages [9,15]. Pressing of coffee pulp yields 14 ˚Brix juice [16], which can evaporate further to make a coffee pulp juice concentrate. According to European Union (EU)' s and Thailand' s Novel Food legislations, coffee cherry skin, and pulp products are considered novel food. EU defines novel food as having no history of "significant" consumption in the European Union before 15 May 1997 [17]. In Thailand, according to Notification No. 376 issued by the Ministry of Public Health, ' novel food' is defined as "any substance used as food or

food ingredients which have been significantly used for human consumption less than fifteen years based on scientific or reliable evidence". Novel food requires scientific assessment of safety before submitting its label to the FDA for approval before use [18]. Information regarding the safety of the novel food such as toxicological studies in animals, including acute studies, sub- chronic studies, and chronic studies or clinical research studies in healthy people are required.

A previous sub-chronic toxicity study in rats reported that the No- observed-adverse- effect level (NOAEL) for the ethanol extract of Coffeeberry® was 4,087 mg/ kg BW/ day [19]. Calculation of acceptable daily intake (ADI) in humans yields 235 mg/ day. Generally Recognized as Safe (GRAS) Notice (GRN) No. 868 of the United States Food and Drug Administration (US FDA) allowed the use of Coffeeberry® Coffee Fruit Extract at levels of up to 300 mg per serving in conventional foods with no premarket approval required [20]. In contrast, an efficacy trial in college athletes reported that consuming 800 mg daily of coffee fruit for 28 days was effective in increasing antioxidant capacity, with no significant adverse effects [21]. Such a finding implied that the safe dose defined by GRAS may not be adequate to provide functional benefit. Nevertheless, there has been no dose-escalation studies in human to determine the maximum safe dose of coffee cherry products. This dose-escalation study aimed to evaluate the safety and tolerability of coffee cherry pulp juice concentrate in healthy subjects, and identify the maximum tolerated dose (MTD).

Materials and Methods

Ethical aspects and setting

The protocol of this study (MU-CIRB 2020/ 329.1510) was approved by Mahidol University Central Institutional Review Board (MU-CIRB), approval number COA No. MU- CIRB2020/ 179. 0511. This research was performed according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice (ICH-GCP) and the Declaration of Helsinki. Informed written consent was obtained from each participant before the study. The protocol was registered in the Thai Clinical Trial Registry (TCTR 20220927004). The protocol can be accessed at https: / / www. thaiclinicaltrials .org/show/TCTR20220927004.

Study design

This is a dose-escalation study with a pre-post design. The study is composed of two phases including a single ascending dose phase and a multiple ascending dose phase. In the single ascending dose phase, a

single dose of coffee cherry pulp juice concentrate was provided once. Then, the next day the dose had been stepwise escalated from 1 to 14 g so the total duration of the study was 14 days. In the multiple ascending dose phase, participants received a stepwise escalating dose from 1 to 14 g of coffee cherry pulp juice concentrate twice daily. Each dose has been repeated for two weeks so the total duration of the study was 12 weeks.

Participants

The inclusion criteria for screening participants of both the single and multiple ascending phases were healthy people aged more than 18 years old who had a body mass index less than 30 kg/ m^2, no systematic diseases e. g. DM, obesity, liver diseases, kidney diseases, thyroid diseases, cancer, and autoimmune diseases, normal vital signs, and normal blood biochemical parameters (complete blood count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, blood urea nitrogen (BUN), creatinine, cholesterol, high- density lipoprotein (HDL), low- density lipoprotein (LDL), triglycerides and fasting plasma glucose (FPG). For the lipid profile, since it is difficult to find participants with normal cholesterol levels, we instead used the Castelli index (the total cholesterol to HDL ratio) for screening. In this study, we included only participants with Castelli risk index-I (Total cholesterol (TC)/ high-density lipoprotein cholesterol; (HDL-C) ≤ 5.0 in men and ≤ 4.5 in women [22]. The exclusion criteria of participants were coagulation problems e.g. hemophilia, idiopathic thrombocytopenia, leukemia, taking anti- coagulants e. g. warfarin, pregnant or plan to be pregnant, breastfeeding, alcohol intake > 14 drinks per week for males or > 7 drinks per week for females and cannot refrain from alcohol intake during the study, smoking > 10 cigarettes/ day, allergy to coffee or caffeine, anemia (Hematocrit < 40 % in males, < 36 % in female or Hemoglobin < 13 g/ dL in males, 12 < g/ dL in females), in critical condition, and having infectious diseases. All participants signed their informed written consent before data collection.

Sample size

The sample size of this study utilized the same number as a previous dose-escalation study for a dietary supplement product [23]. There were 6 participants for the single ascending dose phase and 12 participants for the multiple ascending dose phase. The rationale for the sample size of 6 and 12 comes from the 3+3 principle for clinical safety assessment (phase I clinical trial) to define the maximum tolerated dose (MTD). MTD has been defined as the dose below the dose at which > 33 % of

participants reported adverse events [24-25]. The 3+3 principle stated that if at least one subject among the total subjects of three experienced adverse events, three more subjects should be added to the study [24-25]. Thus, the total number of participants would be six. To follow the principle and ease the data collection, we used 6 participants in the single ascending phase. After receiving a certain dose of coffee cherry pulp juice concentrate, if at least 3 out of 6 participants had adverse events (dose-limiting toxicities), the study would be stopped and the dose below that dose would be the MTD [24-25]. For the multiple ascending dose phase, the MTD was defined as the dose below the dose at which at least 5 out of 12 participants had adverse events [23].

Intervention and materials

Coffee cherry pulp juice concentrate was produced by MiVana Co., Ltd., Samut Prakan, Thailand. Coffee pulp (skin and pulp) is the by-product of coffee bean processing and has been kept frozen. After air thawing for 15-18 h, the coffee pulp was squeezed to yield a juice with 9-13˚ Brix. Then, the juice was evaporated at 55℃ for 16 h to concentrate the juice to 50˚Brix. The juice concentrate was filled in a retort pouch (8 g sachet) and sterilized by water spray retort processing at 100℃ for 30 min. The product passed microbiological and contaminant testing according to the Notification of the Ministry of Public Health (No.355) B.E. 2556 (2013) for the control of food in hermetically sealed containers [26]. Aflatoxin, arsenic, cadmium, lead, mercury, and tin were not detected. It is worth noting that all products used in this study were produced in the same manufacturing batch. Furthermore, the manufacturer also performed quality control to ensure minimal variation between different batches. Per 100 g of the product, there is 0.29 - 0.33% caffeine, 2.7 - 3.00 g dietary fiber, 3.3 - 3.7 g protein, 0.18 - 0.20 mg vitamin E, 34.2 – 37.8 g sugar, 0.17 – 0.19 mg zinc, 0.716 - 0.796 mg copper. Total polyphenol is used for quality control of active compounds, with the range of 449.9 - 497.9 mg eq GA per 100 g of product. The water activity of the product at 25℃ is 0.846 - 0.926. The total antioxidant activity measured by ORAC assay is 9,947.13 - 10,995.13 µmoles TE per 100 g product. In this trial, a coffee cherry pulp juice concentrate beverage was given to participants in a dose-escalation manner. A previous study showed that consuming two capsules of 400 mg of coffee cherry extract powder twice daily (800 mg per day) for 28 days was safe [21]. In this study, the juice has 50˚Brix. Thus, for convenience, we decided to use the starting dose of 1 g which yielded 500 mg of total solid for coffee cherry pulp. In the single ascending phase, participants

received a single dose daily in a stepwise escalation from 1 to 14 g of coffee cherry pulp juice concentrate. Each participant used the 1 g measuring spoon to acquire the assigned dose as shown in Figure 1. For example, one spoon for 1 g, two spoons for 2 g, …, and 14 spoons for 14 g. Participants consumed each dose only once. For multiple ascending doses, participants consume a stepwise escalating dose from 1, 4, 8, 10, 12, and 14 g of coffee cherry pulp concentrate twice daily (2, 8, 20, 24, and 28 g per day). Each dose has been repeated for two weeks. Each participant used the 1 g, 2 g, 8 g, and 10 g measuring spoon to acquire the assigned dose as shown in Figure 2.

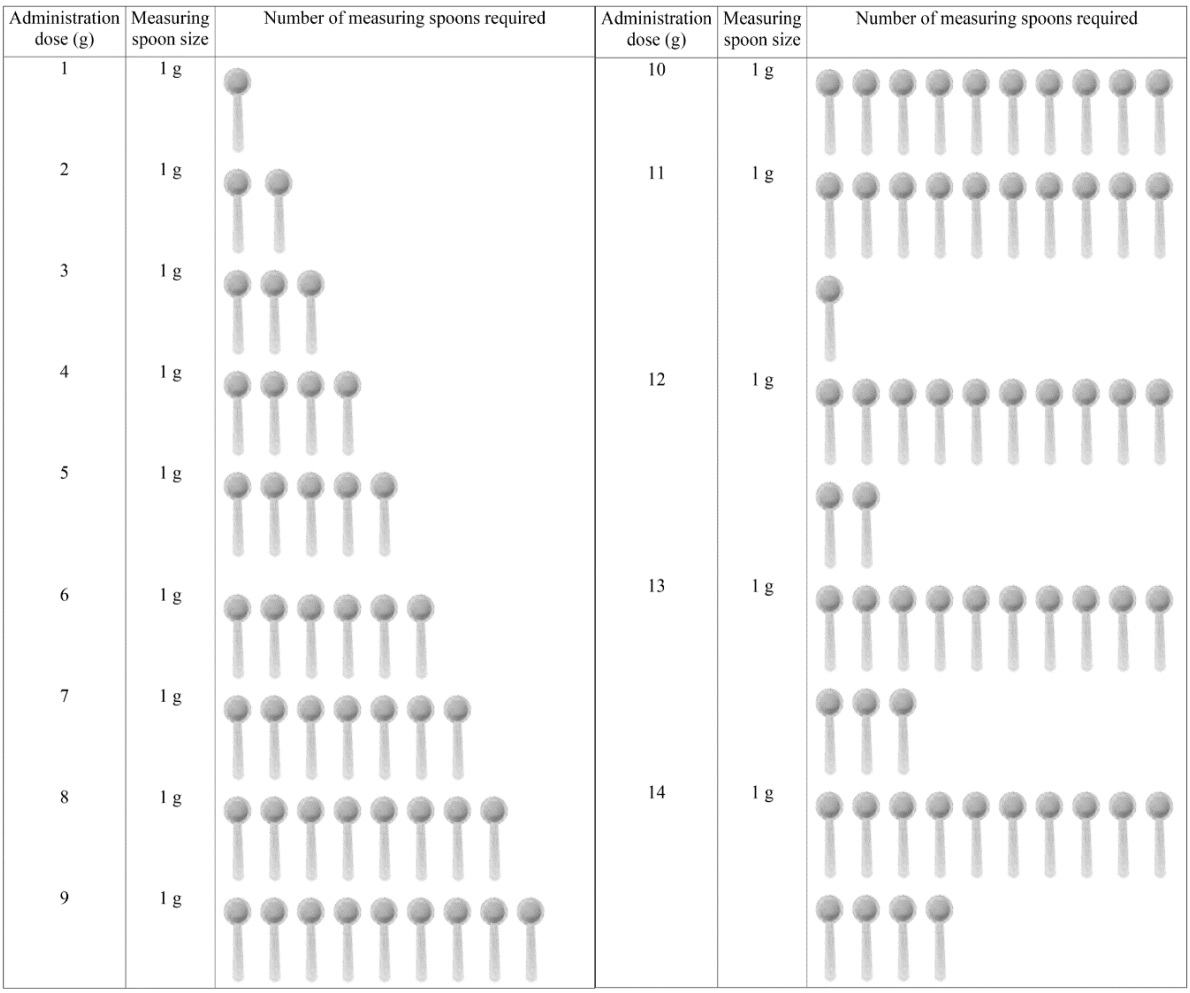


Figure 1. Diagram of intervention for single ascending dose phase

The diagram shows the number of 1 g measuring spoons (gray spoon icon) for each dose. During this phase, the participant consumed each assigned dose once daily for one day and step-wise escalated to a higher dose as shown.

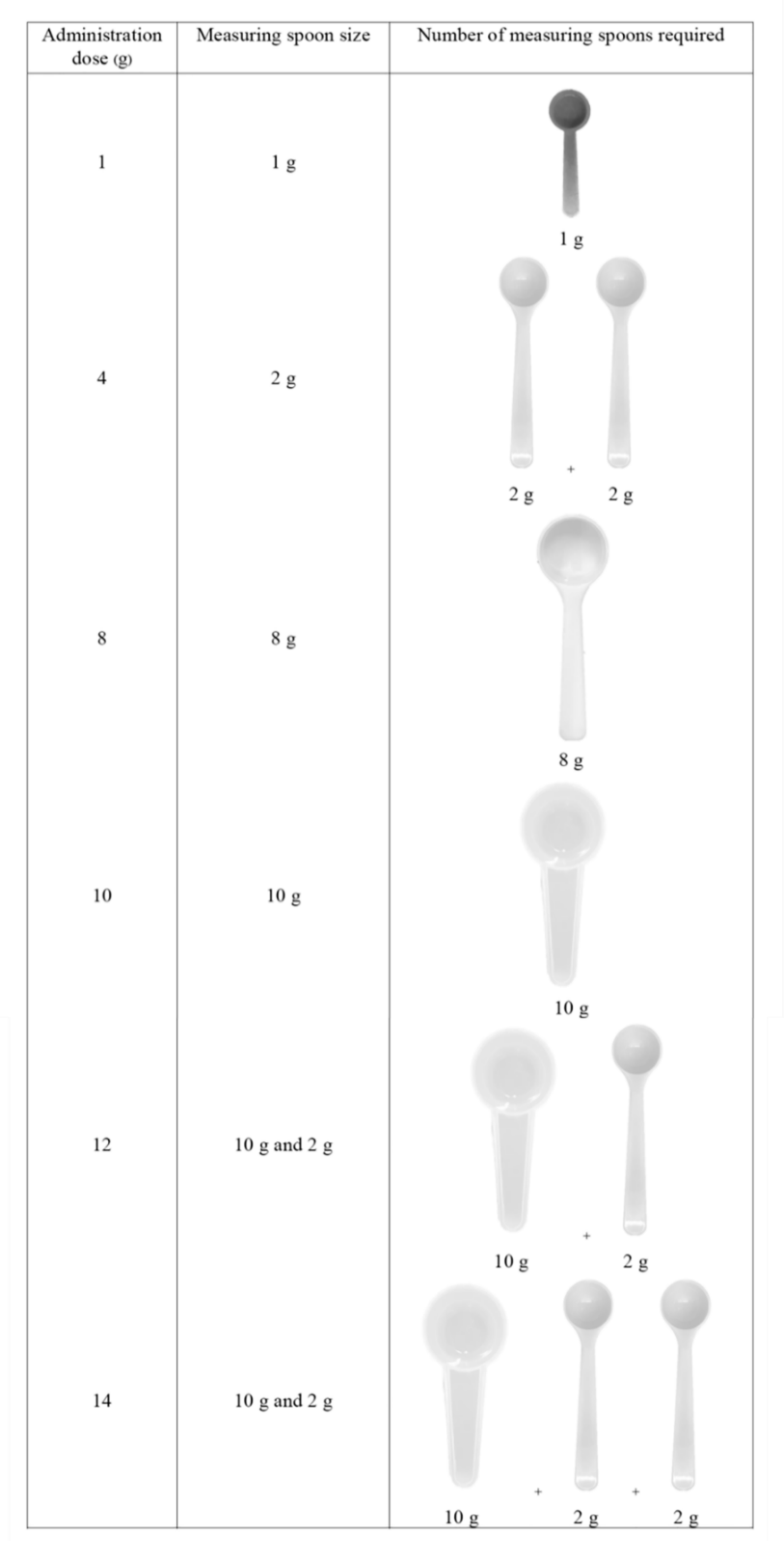


Figure 2. Diagram of intervention for multiple ascending dose phase

The diagram shows the type and number of measuring spoons for each assigned dose. During this phase, the participant consumed each assigned dose twice daily for two weeks and step-wise escalated to a higher dose as shown.

Study procedure and outcome measurement

This single-arm, monocentric, dose-escalation study was performed on healthy participants. Dose escalation was carried out in two phases including the single ascending dose (SAD) phase, and the multiple ascending (MAD) phase.

- ***Single ascending dose (SAD)***

The study flow scheme of the SAD phase is presented in Figure 3. The safety and tolerability of a single dose of coffee pulp juice concentrate were investigated in six participants. All participants consume each assigned dose of the product once a day for 1 day each and record their symptoms in a subject diary. They were also asked to make a diet record of the food they consumed every day. All participants were asked to avoid taking any dietary supplements, and alcoholic beverages throughout the study. For participants who regularly drink coffee, we asked them to consume it at least 1-2 hours apart from consuming the product. All participants were asked to call us immediately after they had any serious abnormal symptoms. After taking the first dose, if no adverse events or adverse effects were occurring in no more than 2 subjects, participants could step up to the higher assigned dose until the end of the study. However, if at least 3 subjects had adverse symptoms, the study would be terminated. And the maximum tolerated dose (MTD) is the previous safe dose level. The SAD started at 1 g and escalated to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 g. The total study duration was 14 days.

There were three visits for all participants including visit 1 for screening, visit two for starting product delivery, and visit three for follow-up after 14 days. In visit 1 (V1), recruited volunteers were screened according to the inclusion and exclusion criteria. History taking for medical status and medication/ dietary supplement use were performed. Vital signs (blood pressure, and heart rate), body weight, and blood biochemical parameters (complete blood count, fasting blood glucose, lipid profile, liver, and kidney function biomarkers) were measured. In visit 2 (V2), coffee pulp juice concentrate products were given to all participants with instructions to consume one assigned dose per day and how to make records of adverse events, assigned product intake, and other food consumption. In visit 3 (V3), participants returned for a follow-up of adverse symptoms and returned the empty sachets for a compliance check. Body weight and vital signs were measured.

Visit 1 Screening: collection of demographic parameters (age, body weight, BMI), vital signs, blood biochemical values)

-------- Screening period, 4-7 days --------

Visit 2 Product delivery: Issues of coffee cherry concentrate product and subject diaries

Administration period: Subject receive coffee cherry concentrate in following dosages & timeframes

Duration (days)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Dose (g)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Frequency	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily	Once daily
Total number subjects	6	6	6	6	6	6	6	6	6	6	6	6	6	6
0-2 subjects with adverse events	**Escalation to next dose**													MTD is 14 g
≥3 subjects with adverse events	Stop	Stop, MTD is 1 g	Stop, MTD is 2 g	Stop, MTD is 3 g	Stop, MTD is 4 g	Stop, MTD is 5 g	Stop, MTD is 6 g	Stop, MTD is 7 g	Stop, MTD is 8 g	Stop, MTD is 9 g	Stop, MTD is 10 g	Stop, MTD is 11 g	Stop, MTD is 12 g	Stop, MTD is 13 g

Visit 3 Follow-up after 14 days: Collection of subject diaries, interview for adverse events, body weight, and vital signs

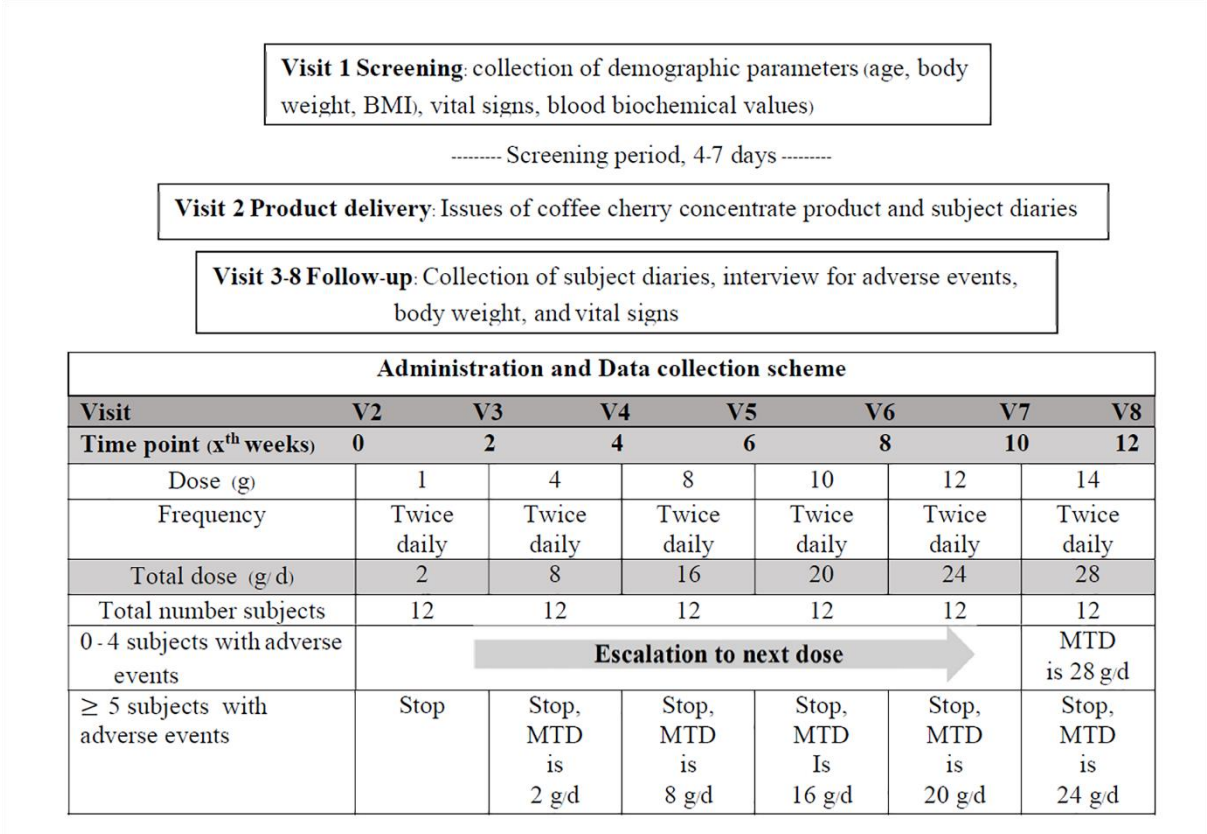
Figure 3. The study flow scheme of the single ascending dose (SAD) phase

BMI, body mass index; MTD, maximum tolerated dose

• ***Multiple ascending doses (MAD)*** After the SAD phase finished, repeated doses for MAD phase were selected based on the SAD phase. The study flow scheme of the MAD phase is presented in Figure 4. The safety and tolerability of the repeated doses of coffee pulp juice concentrate were investigated in twelve participants. All participants consume each assigned dose of the product twice a day for two weeks and record their symptoms in a subject diary. They were also asked to make a diet record of the food they consumed three days per week (two weekdays and one weekend day). All participants were asked to avoid taking any dietary supplements, and alcoholic beverages throughout the study. For participants who regularly drink coffee, we asked them to consume it at least 1-2 hours apart from consuming the product. All participants were asked to call us immediately after they had any serious abnormal symptoms. After taking the first dose, if there were no adverse events or adverse effects happened in no more than 4 subjects, participants could step up to the higher assigned dose until the end of the study. However, if at least 5 subjects had adverse symptoms, the study would be terminated. And the maximum tolerated dose (MTD) is the previous safe dose level. The MAD started at the dose of 1 g twice daily (2 g per day) repeatedly for two weeks and escalated to 4, 8, 10, 12, and 14 g twice

daily (8, 20, 24, and 28 g per day) repeatedly for two weeks each. The total study duration was 12 weeks.

There were eight visits for all participants including visit 1 for screening, visit 2 for starting product delivery, and visit 3-8 for follow-up after two weeks of consumption for each dose. In visit 1 (V1), recruited volunteers were screened according to the inclusion and exclusion criteria. History taking for medical status and medication/ dietary supplement use were performed. Vital signs (blood pressure, and heart rate), body weight, and blood biochemical parameters (complete blood count, fasting blood glucose, lipid profile, liver, and kidney function biomarkers) were measured as baseline parameters. In visit 2 (V2), coffee pulp juice concentrate products were given to all participants with instructions to consume one assigned dose repeatedly for two weeks and how to make records of adverse events, assigned product intake, and other food consumption. In visits 3-8 (V3-V8), participants returned for follow- up of adverse symptoms and returned the empty sachets for a compliance check. Body weight, vital signs, and blood biochemical parameters were measured and compared with the baseline.



Administration and Data collection scheme

Visit	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**
Time point (x^{th} weeks)	**0**	**2**	**4**	**6**	**8**	**10**	**12**
Dose (g)	1	4	8	10	12	14	
Frequency	Twice daily	Twice daily	Twice daily	Twice daily	Twice daily	Twice daily	
Total dose (g/d)	2	8	16	20	24	28	
Total number subjects	12	12	12	12	12	12	
0 - 4 subjects with adverse events	**Escalation to next dose**					MTD is 28 g/d	
≥ 5 subjects with adverse events	Stop	Stop, MTD is 2 g/d	Stop, MTD is 8 g/d	Stop, MTD Is 16 g/d	Stop, MTD is 20 g/d	Stop, MTD is 24 g/d	

Figure 4. The study flow scheme of multiple ascending doses (MAD) phase

BMI, body mass index; MTD, maximum tolerated dose

Statistics

Characteristics of participants were summarized as mean and standard deviation (SD) for numerical data, and frequency and percent for categorical data. Adverse events were counted and displayed as percentages. The body weight and blood biochemical parameters after consuming doses of coffee cherry juice concentrate were compared with baseline by using Kruskal-Wallis tests, followed by Dunn's multiple comparison tests. All statistical tests were performed by using a two-tailed test. P-value < 0.05 was considered statistically significant. Graph Pad Prism V.9.3 was used for graphing and statistical analysis.

Results

Single ascending dose (SAD)

- ***Characteristics of participants***

A total of 6 healthy participants (3 males and 3 females) were enrolled in this study. The mean ± SD age of the subjects was 29.33 ± 4.03 years old, ranging from 25 - 35 years. The mean BMI of the study subjects was 18.30 ± 0.73 kg/ m^2, ranging from 18.31 – 20.21 kg/ m^2. All subjects had no histories of past and present illness, smoking, and drinking, and met the inclusion criteria. All subjects completed the study. The demographic characteristics of the enrolled subjects are summarized in Table 1.

- ***Adverse event***

As shown in Table 2, during the SAD phase, there were no adverse events reported by the subjects.

- ***Maximum Tolerated Dose (MTD) for single intake***

The maximum tolerated dose for a single intake of coffee cherry pulp juice concentrate was 14 g for single use, which is the highest dose that no adverse events were observed.

Multiple ascending doses (MAD)

- ***Subject demographics***

A total of 12 healthy subjects (3 males and 3 females) were enrolled in this study. The mean ± SD of age was 34.67 ± 7.08 years, ranging from 26 to 49 years. The mean ± SD of BMI was 22.21 ± 4.13 kg/ m^2, ranging from 18.31 to 30.30 kg/ m^2. All subjects had no history of past and present illness, smoking, and drinking, and met the inclusion criteria. No subjects withdrew from this study. The demographic characteristics of the enrolled subjects are summarized in Table 3.

- ***Adverse event***

As shown in Table 4, the coffee cherry pulp juice concentrate was administered orally twice daily for 14 days each dose tested was generally safe and well-tolerated, with no adverse events.

- ***Body weight change***

During the MAD phase, there were no relevant significant changes in body weight during coffee cherry pulp juice concentrate intake (**Figure 5)**.

- ***Clinical chemistry and hematology test***

As shown in Table 5, throughout the MAD phase after consuming coffee cherry juice concentrate from the dose of 2 to 28 g, there were no significant changes in the average fasting blood glucose, HDL, eGFR, BUN, WBC, RBC, hemoglobin, hematocrit, MCV, MCH, RCDW, and platelet count. Compared to baseline values, the average levels of total cholesterol, LDL, and creatinine were significantly decreased ($p < 0.05$) after consuming the product at a dose of 20 g/ day. This is considered a favorable effect. In contrast, starting from the dose of 8 g per day the average levels of AST and ALT (liver biomarkers) were significantly increased ($p < 0.05$). However, all average values were still much lower than those of the normal limit of 40 U/ L. Thus, the slight increase in liver markers is not considered liver toxicity. Likewise, starting from the dose of 16 g per day the average levels of MCHC were significantly increased ($p < 0.05$). However, the increased values were in a normal range, suggesting no toxicities.

As shown in Table 6. It is worth noting that there were some participants with increased liver markers (AST and ALT) exceeding the normal limit of 40 U/ L during the MAD phase. History- taking revealed that some participants took some herb (King of bitters (*Andrographis paniculata* (Burm.f.) Wall. ex Nees) or drug (antihistamine: cetirizine) to ease the common cold. Moreover, a decrease in WBC, hemoglobin, or hematocrit was observed in some participants, which may be correlated with their low- normal values of hematological parameters at baseline, blood loss from menstruation, and some accidental supplement intakes during enrolling in this study. However, these slightly abnormal clinical laboratory values were observed in less than 33% of all subjects in each dosage and the values of liver enzymes and hematological values in those subjects were resumed to the normal range at the higher dosages of coffee cherry pulp juice concentrate intake. Thus, all these changes were likely not related to the intake of coffee cherry pulp juice concentrate.

- ***Maximum Tolerated Dose (MTD) for repeated intake***

Since the number of participants with abnormal biochemical parameters was not exceeding 33% throughout the study, the highest dose in this study (14 g coffee cherry pulp juice concentrate twice daily or 28 g total per day becomes the maximum tolerated dose (MTD).

Table 1. Demographic characteristics of participants in single ascending phase

Parameter	SAD (n = 6)
Gender, n (%)	
Male	3 (50)
Female	3 (50)
Age (years)	
Mean ± SD	29.33 ± 4.03
Range (min – max)	25 - 35
BMI (kg/ m^2)	
Mean ± SD	18.30 ± 0.73
Range (min-max)	(18.31 - 20.21)
Systemic diseases, n (%)	0 (0)
Smoking, n (%)	0 (0)
Alcohol drinking, n (%)	0 (0)

SAD, single ascending dose; BMI, body mass index

Table 2. Summary of adverse events in the single ascending dose (SAD) phase

Variables	Coffee cherry concentrate dose (g/ day)													
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Subjects with any adverse events	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Specific adverse events														
Burning mouth	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nausea	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vomiting	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abdominal pain	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Headache	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* Data are shown as the number of subjects with adverse events

Table 3. Demographic characteristics of MAD subjects

Parameter	MAD (n = 12)
Gender, n (%)	
Male/ Female	6 (50)/ 6 (50)
Age (years)	
Mean ± SD	34.67±7.08
Range (min, max)	(26, 49)
BMI (kg/m^2)	
Mean ± SD	22.21±4.13
Range (min, max)	(18.3, 30.30)
Illness, n (%)	0
Smoking, n (%)	0
Drinking, n (%)	0

MAD, Multiple ascending dose; BMI, body mass index.

Discussion

Previously viewed as industrial waste in coffee manufacture [9], coffee cherry pulp now receives attention as a functional food with potential health benefits [8-11]. Products made from coffee cherry pulp are considered novel food, which requires safety assessment prior to approval by regulators [17-18]. However, the maximum safe dose of this novel ingredient for human consumption was unknown**.** This dose-escalation study reports the maximum tolerated dose (MTD) of coffee cherry pulp juice concentrate at the dose of 14 g for a single intake and 28 g per day for two weeks of repeated intake**.** The dose can be used to further study the long**-** term safety of repeated dose consumption**.**

Previous toxicological studies for coffee cherry products include the ethanolic extract (alcohol extraction) of Coffeeberry® whole coffee fruit. However, no studies had been reported on the safety of coffee cherry pulp juice (directly squeezed liquid from the pulp). The present study is the first report to suggest that healthy people can safely consume coffee cherry pulp juice concentrate up to 28 g per day for up to two weeks. The 50˚Brix coffee pulp juice concentrate used in this study contained 50% total soluble solid. Thus, the MTD of 28 g juice concentrate has a soluble

solid of 14 g/ day. Such an amount could also be applied to design further safety testing of other coffee pulp- derived products. In a previous animal study [19], the calculated safe dose of the ethanol extract of Coffeeberry® whole coffee fruit is 235 mg/ day, while our present study yields a much higher safe dose of 28 g/ day. The reasons for this tremendous difference include the limitation of dose administration in animals and the existence of organic solvents. The coffee cherry pulp juice reported in the present study was produced by direct squeezing the liquid from the pulp with no addition of organic solvent. Therefore, the contents of certain bioactive compounds in the juice are likely lower but the safety is better than the ethanolic extract. In fact, the coffee pulp juice concentrate in our study has total antioxidant activity (oxygen radical absorption capacity: ORAC assay) of 99.47 – 109.95 µmoles TE per 1 g product. The previous study of Coffeeberry® whole coffee fruit extract reported the total antioxidant activity (ORAC assay) of 6,000 µmoles TE per g for ethanolic extract or 1500 µmoles TE per g for a water extract or 800 µmoles TE per g for whole powder [19]. Compared with those reported in the previous study, the coffee pulp juice in our present study has much lower antioxidant activity, suggesting lower content of bioactive compounds. Furthermore, the caffeine content in coffee pulp juice is 0.29 - 0.33 %, while those of ethanolic extract, water extract, and whole powder of Coffeeberry® are 0.6 - 9.08 %, 1 %, and 0.7 – 1 % respectively. The lower amount of active compounds and the solvent- free nature of the juice may explain the superior safety of coffee pulp juice concentrate. Also, the lower caffeine content of the juice makes it safer to consume for people sensitive to caffeine.

Table 4. Summary of adverse events in the multiple ascending dose (MAD) phase

Variables	Coffee cherry concentrate dose (g/ day)					
	2	**8**	**16**	**20**	**24**	**28**
Subjects with any adverse events	0	0	0	0	0	0
Specific adverse events						
Burning mouth	0	0	0	0	0	0
Nausea	0	0	0	0	0	0
Vomiting	0	0	0	0	0	0
Abdominal pain	0	0	0	0	0	0
Headache	0	0	0	0	0	0

* Data are shown as the number of subjects with adverse events

Table 5. Changes in blood parameters after consumption of 2 - 28 g per day of coffee cherry pulp juice concentrate

Parameter (unit)	**Normal range**	**Baseline**	**2 g/ day**	**8 g/ day**	**16 g/ day**	**20 g/ day**	**24 g/ day**	**28 g/ day**
Fasting blood glucose (mg/dL)	70 - 110	101.3 ± 6.4	108.3 ± 6.6	102.4 ± 6.2	99.2 ± 8.3	97.8 ± 3.8	96.7 ± 8.3	97.6 ± 9
Total cholesterol (mg/dL)	< 200	250 ± 43.4	234.5 ± 29.2	225.7 ± 41.2	222.6 ± 36.4	202.3 ± 23.6 **	211 ± 27.7*	206.1 ± 35.9 *
HDL cholesterol (mg/dL)	> 45	60.08 ± 7.9	63 ± 7.5	64.75 ± 7.5	64.17 ± 8.6	64.7 ± 8.4	67.3 ± 8.3	63 ± 8.9
LDL cholesterol (mg/dL)	< 130	166.8 ± 39.9	149.8 ± 29.3	140 ± 40.05	139.8 ± 35.3	119.7 ± 22.5 **	125.6 ± 28.9*	128.8 ± 32.7
BUN (mg/dL)	6 - 20	10.83 ± 2.9	11.9 ± 2.6	12.67 ± 3.02	12.72 ± 2.8	13.33 ± 2.9	12.5 ± 2.99	12.4 ± 3.1
Creatinine (mg/dL)	0.5 - 1.5	0.82 ± 0.09	0.82 ± 0.1	0.76 ± 0.06	0.74 ± 0.08	0.69 ± 0.08 **	0.73 ± 0.1	0.73 ± 0.1
eGFR (ml/min/1.73 m^2)	> 90	109 ± 7.9	108.4 ± 9.3	114.1 ± 6.3	116.6 ± 9.8	118.5 ± 9.6	114.1 ± 9.6	113.9 ± 8.6
AST (U/L)	0 - 40	16.17 ± 7.8	15.5 ± 6.1	24.58 ± 6.7 *	24.17 ± 7.01	22 ± 9.6	24.8 ± 7.1	21.4 ± 5.3
ALT (U/L)	0 - 40	12.25 ± 6.9	11.92 ± 7.7	18.17 ± 6.5 *	18.58 ± 7.8 *	19.08 ± 8.03 *	21.8 ± 11.9	19.3 ± 10.2
WBC (cells/ cu.mm)	4,000 - 10,000	6.558 ± 1.7	6.483 ± 1.7	6.5 ± 1.9	6.473 ± 1.3	5.98 ± 1.6	6.86 ± 1.7	6.36 ± 1.5
RBC (million cells/ cu.mm)	Male: 4.5 - 6.0 Female: 4.0 – 5.5	4.96 ± 0.39	4.803 ± 0.4	4.774 ± 0.4	4.628 ± 0.4	4.7 ± 0.4	4.7± 0.39	4.8 ± 0.4
Hemoglobin (g/ dL)	Male: 13.0 - 18.0 Female: 12.0 - 16.0	13.71 ± 1.35	13.33 ± 1.4	13.33 ± 1.6	13.21 ± 1.5	13.02 ± 1.5	13.03 ± 1.4	13.5 ± 1.6
Hematocrit value (%)	Male: 40 - 54, Female: 36- 58	41.67 ± 3.4	39.67 ± 3.6	39.92 ± 4.7	38.83 ± 4.02	38.8 ± 3.9	39.1 ± 3.8	39.2 ± 4.4
MCV (fL)	80.0 - 99.0	83.08 ± 4.8	81.72 ± 4.4	82.71 ± 4.3	82.55 ± 4.6	82.1 ± 4.5	83.1± 4.2	81.2 ± 4.5
MCH (pg)	27.0 - 31.0	27.48 ± 2.1	27.5 ± 1.9	27.61 ± 1.8	28.18 ± 1.8	27.5 ± 1.9	27.6 ± 2	27.9 ±1.9
MCHC (g/dL)	33.0 - 37.0	33.06 ± 0.8	33.62 ± 0.8	33.36 ± 0.6	34.1 ± 0.6 *	33.5 ± 0.8	33.2 ± 1	34.4 ± 0.7 **
RCDW (%)	11.0 - 14.5	13.88 ± 1.8	13.83 ± 1.9	13.68 ± 1.8	13.88 ± 1.5	13.44 ± 1.2	13.54 ± 1.3	13.66 ± 1.4
Platelet count (cells/ cu.mm)	140,000 - 450,000	30,6417 ± 43	308,417 ± 50,891	301,583 ± 51,205	329,750 ± 64,373	321,917 ± 49,144	323,417 ± 67,629	305,000 ± 55,793

Data are shown as mean ± SD. * and ** represent $p < 0.05$ and $p < 0.01$, respectively, compared to baseline values. HDL, high-density lipoprotein; LDL, low-density lipoprotein; BUN, Blood Urea Nitrogen; eGFR, estimated Glomerular Filtration Rate; AST, Aspartate transaminase; ALT, Ala-nine aminotransferase; WBC, White blood cell count; RBC, Red blood cell count; MCV, Mean cell volume; MCH, Mean cell hemoglobin; MCHC, Mean cell hemoglobin concentration; RDW, Red blood cell distribution width.

Table 6. Number of subjects with abnormal clinical laboratory values during coffee cherry pulp juice concentrate intake

Dosage	Abnormal clinical laboratory	N, (%)	Abnormal values	Related History	Dose-response [$]
2 g/ day	Hematocrit decreased	1, (8.33)	33% (Normal: 36 - 58%)	Low normal at baseline + Menstruated	No
8 /gday	Hematocrit decreased	1, (8.33)	33% (Normal: 36 - 58%)		No
16 g/ day	AST increased	1, (8.33)	44 U/L (Normal: 0 - 40 U/ L)	Herb intake	No
	Hematocrit decreased	1, (8.33)	32 % (Normal: 36 - 58%)	Low normal at baseline + Menstruated	No
20 g/ day	AST increased	1, (8.33)	48 U/L (Normal 0 - 40 U/L)	Cetirizine drug intake	No
	WBC decreased	1, (8.33)	3,300 cells per cc. (4,000 - 10,000 cells/ cu.mm)	Low normal at baseline	No
	Hematocrit decreased	1, (8.33)	32 % (Normal: 36 – 58%)	Low normal at baseline + Menstruated	No
24 g/ day	ALT increased	1, (8.33)	47 U/L	Herb intake	No
	Hemoglobin decrease	1, (8.33)	11.3%	Low normal at baseline +herbal tea intake	No
	Hematocrit decreased	1, (8.33)	32%		
28 g/ day	ALT increased	1, (8.33)	47 U/L	Herb intake	No
	Hemoglobin decrease	1, (8.33)	11.3%	Low normal at baseline + herbal tea intake	No
	Hematocrit decreased	1, (8.33)	29%		No

[$] Dose-response relation means having more changes with increasing dose. N = number of participants

A previous clinical trial showed that consumption of whole coffee fruit powder at 800 mg per day for 4 weeks increased antioxidant capacity in college athletes, with no adverse event effects [21]. An animal study demonstrated that water extract of Coffeeberry® improved the lipid profile in hypercholesterolemic rats [14]. Consistently, another animal study showed that intake of Coffeeberry® whole coffee fruit powder reduced lipid peroxidation products in the liver of rats with non-alcoholic fatty liver [27]. Nevertheless, the efficacy of coffee pulp juice concentrate on health has never been reported. In the present study, we found a significant decrease in the level of total cholesterol and LDL after consuming at least 20 g of coffee cherry pulp juice concentrate for two weeks. In this study, almost all participants have total cholesterol over 200 mg/ dL and LDL over 130 mg/ dL but their Castelli index values (TC/ HDL ratio) were in the normal range. Therefore, this group of people may be considered mild

dyslipidemia. Previous studies suggested that the reduction of total cholesterol levels and LDL levels can reduce the risk of coronary heart disease [28-29]. Thus, coffee cherry pulp juice concentrate might have a potential lipid-lowering effect and heart-health promotion. Nevertheless, two weeks period in this study was too short to conclude the lipid-lowering effect. A future clinical trial with a continuous intake of coffee pulp juice concentrates for at least 4 weeks as recommended [30] is warranted.

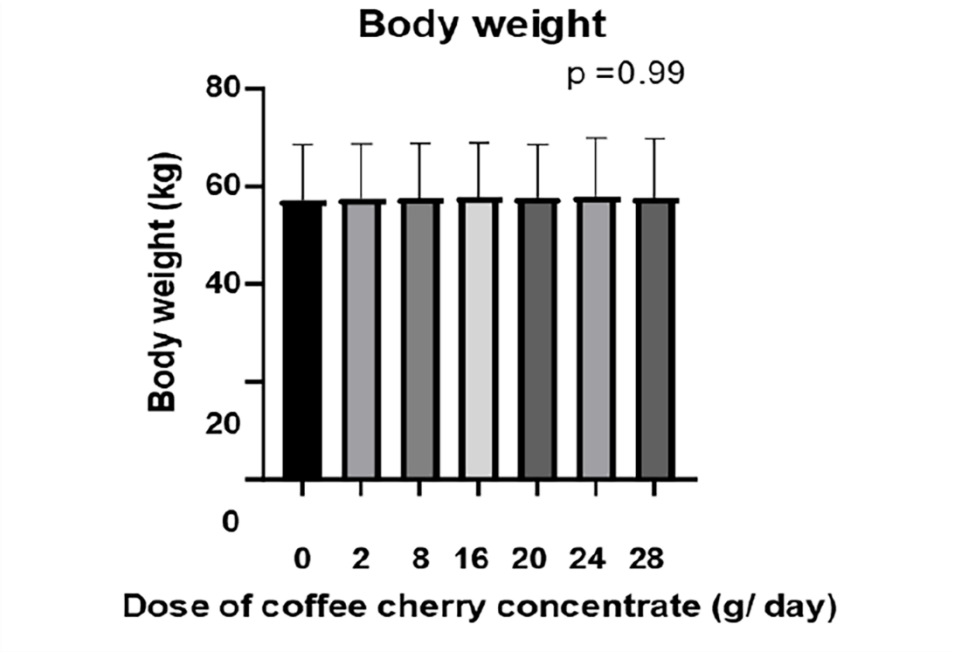


Figure 5. Changes in body weight before and after coffee cherry pulp juice concentrate intake. The bar graph shows the mean ± SD of body weight in all participants (n = 12) at baseline (0), and after consuming 2, 8, 16, 20, 24, and 28 g of coffee cherry juice concentrate per day, as specified**.** The p-value was obtained from Kruskal - Wallis test.

In this study, we found a transient increase in liver enzymes of some participants during the period that the participants used some herbs and drugs. However, when they stop using the drugs, their liver enzymes returned to normal. Therefore, the rise in liver enzymes is likely not relevant to the investigational product we provided. Nevertheless, the product contains phytochemicals, which could be metabolized via the liver detoxification system and the liver function may be more loaded after product consumption. Thus, future clinical trials should closely monitor liver function.

The strength of this work includes the step-wise dose-escalation which allows us to monitor the participants' adverse events periodically and the good compliance of all participants (no drop-out). The limitations of this study were the short duration of repeated intake (14 days) in the multiple-

ascending dose study and the lack of a placebo control group to compare. A future randomized placebo control trial for repeated doses of coffee pulp juice concentrate for a longer duration is warranted.

Conclusion

This study suggests that the consumption of coffee cherry pulp juice concentrate up to 28 g daily for two weeks is safe in healthy subjects. A future long-term randomized, placebo-controlled trial is required to confirm our findings.

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Conflicts of Interest

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Data Availability Statement

The data presented in this study are available upon reasonable request to the corresponding author**.**

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