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Hesperetin-7-Glucoside-β-Cyclodextrin Inclusion Complex is Associated with Improvement in Vascular Endothelial Function, and Mental and Physical Health in Healthy Subjects:

A Randomized, Parallel, Double-Blind, and Placebo-Controlled Study

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● **Abstract**

Objective: The purpose of this study was to assess the impact of hesperetin-7-glucoside-inclusive cyclodextrin (HCD) intake on endothelial dysfunction (ED) and the mental and physical health of participants.
Methods: Healthy adult male and female subjects were enrolled in a randomized, parallel, double-blind, placebo-controlled study. Participants consumed either 150 mg or 300 mg of HCD after breakfast and dinner for twelve consecutive weeks compared with the placebo. ED was evaluated using FMD scores measured by an FMD monitoring device at baseline (0 w) and at twelve weeks (12 w). Mental and physical effects were assessed through changes in Visual Analog Scale (VAS) scores from 0 w to 12 w.
Results: Among the 59 subjects, after 300 mg HCD (300H) intake showed a significant improvement in FMD at 12 w compared to the placebo (0H) group, while 150 mg HCD (150H) intake did not reach statistical significance. In addition, 300H intake significantly alleviated weariness, dark circles under the eyes, and eyelid swelling compared to the placebo group, while 150H intake did not demonstrate significant effects. No clinically relevant or biochemically adverse effects were observed during the study.

Keywords: Hesperetin-7-Glucoside, Cyclodextrin, Flow-mediated vasodilation, Weariness, Dark circles under the eyes

1. INTRODUCTION

Blood vessels have a three-layered structure, consisting of an intima composed of vascular endothelial cells in the innermost layer of the vascular wall, a media containing smooth muscle cells, and an adventitia[1]. Vascular endothelial cells play a crucial role in maintaining body homeostasis by producing vascular regulatory factors, including nitric oxide (NO) and endothelin (peptide), which regulate vascular wall expansion and contraction[2]. Measurement of flow-mediated vasodilation (FMD), analyzing the change (%) in artery diameter, has been utilized to assess vascular endothelial function[3)-5)]. FMD serves as an indicator for the progression of arteriosclerosis associated with dyslipidemia, hypertension, diabetes, obesity, and cardiovascular disease[6)-8)].

While some studies have reported FMD levels above 6-8% as normal values[9)-11)], many reports indicate a decline in FMD with factors such as age, smoking, and body mass index (BMI)[10)12)-13)]. Dyslipidemia has been linked to endothelial dysfunction. However, the relationship between components of a lipid profile, such as low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL), triglycerides (TG), and endothelial function, is not straightforward[14)]. Thus, the complexity of the FMD score arises due to its association with factors like gender, age, smoking, BMI, as well as plasma components such as LDL, HDL, and TG[10)13)-15)].

Polyphenols (PPs), found in citrus fruits, green tea, and red wine, are promising plant compounds known for their antioxidant potential and significant improvement effects on endothelial dysfunction[16)-18)]. Certain PPs, including chlorogenic acid, rutin, and hesperidin have shown notable improvements in skin smoothness, leg swelling, and fatigue due to increase NO production in vascular endothelial cells and enhanced blood flow[19)-21)].

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Table 1 Analysis of the nutrient composition of the test food※

Description	0H	150H	300H
Energy (kcal)	1.51	1.52	1.52
Protein (mg)	−	0.42	0.84
Fat (mg)	6.3	8.4	8.4
Carbohydrate (mg)	364.14	360.78	360.36
Salt equivalent (mg)	0.01	5.3	10.4
HCD (mg)	0	150	300
HPTG (mg)	0	19.5	39.0

※0H, Placebo; 150H, HCD 150 mg/day; 300H, HCD 300 mg/day.

Some groups have demonstrated the broad pharmacological activities of Hesperidin (HSP; hesperetin-7-rutinoside), a flavonoid abundantly present in citrus fruit peels, due to its antioxidant and anti-inflammatory properties. Intake of HSP at a dosage range of 159.5 mg to 1g per day for 1-6 weeks has shown improvements in FMD impairment, metabolic syndrome, cardiovascular health, atherosclerosis, and systolic blood pressure (SBP) compared to the placebo[22)-26)].

The potential mechanism underlying the improvement in endothelial dysfunction, atherosclerosis, and SBP following HSP intake can be explained as follows:

1) HSP stimulates NO production in endothelial cells, leading to vasorelaxation, improved blood flow, and endothelial regeneration[3)24)27)].
2) HSP inhibits reactive oxygen species (ROS) production in various cell types, thereby reducing vascular oxidant stress[28)-30)].
3) HSP acts as a direct vasodilator by stimulating K^+ channels and inhibiting Ca^{+2} channels[31)-33)].

However, the limited bioavailability of HSP, which is absorbed in the large intestine after hydrolysis by colonic microflora, restricts its broad pharmacological activities. On the other hand, Hesperetin-7-glucoside (HPTG) exhibits several times higher area under the curve (AUC) than HSP due to efficient absorbance in the small intestine after hydrolysis by lactase phloridzin hydrolase (LPH) in the brush border of small intestine epithelial cells[34)-36)].

In our recent study involving healthy adult male participants, we reported that administration of hesperetin-7-glucoside-β-cyclodextrin inclusion complex (HCD) resulted in significantly improved bioavailability compared to HSP. The AUC_{0-24}, maximum concentration (Cmax), and time to Cmax (Tmax) of HCD were approximately 100-fold greater, nearly 440-fold higher, and 6 hours shorter, respectively, than those of HSP[36)]. Furthermore, their cyclodextrin-inclusive structures of HCD[37)] and its safety profile, as determined by a 90-day subchronic toxicity study (No Observed Adverse Effect Level; NOAEL): 3267.7 mg/kg/day, equivalent to 464 mg/kg/day as HPTG conversion for males; 3652.4 mg/kg/day, equivalent to 519 mg/kg/day as HPTG conversion for females), have also been reported[38)].

Based on these findings, we formulated the HCD complex and conducted a 12-week placebo-controlled, double-blind, parallel-group comparative study in healthy subjects. Participants were administered 150H, 300H, or placebo (0H), and their FMD levels were evaluated using an FMD monitoring device. Additionally, their mental and physical health was assessed using the Visual Analog Scale (VAS).

2. MATERIALS AND METHODS

2.1. Ethical approval of the study protocol

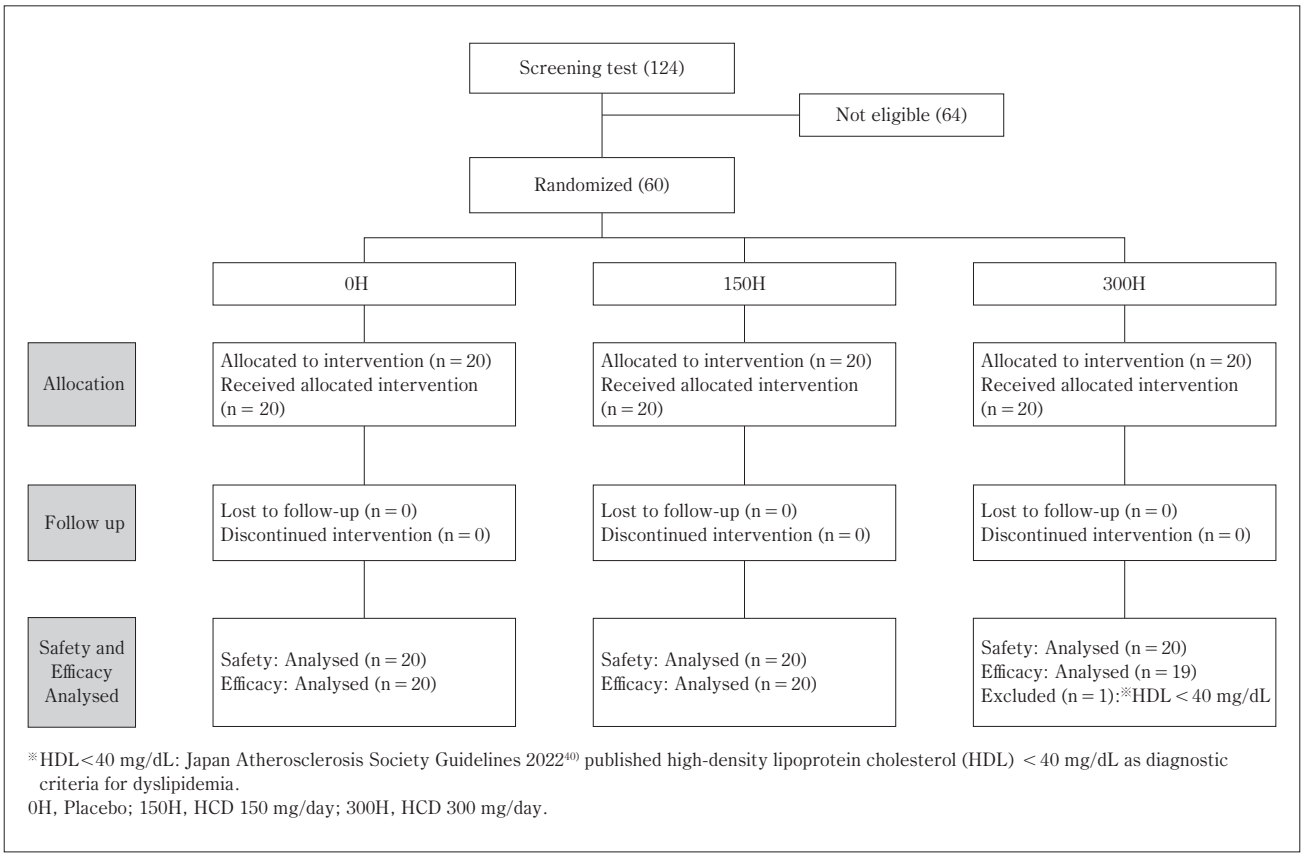
The study was conducted in accordance with the Helsinki Declaration based on the "Ethical Guidelines for Medical and Health Research Involving Human Subjects" (Notification by the Ministry of Health, Labor and Welfare, partially revised on February 28, 2017). The Suda Clinic institutional review board approved the study protocol (Approval number: 2022-018; Date of approval: June 21, 2022). The study protocol was registered at the UMIN-CTR (Trial ID: UMIN000048342). The protocol was not modified from the time of the final setup and during the study.

2.2. Supplementation and sample size

Hard capsules including HCD (SUNACTIVE® HCD/HES, Taiyo Kagaku Co., Ltd., Japan) were prepared for the study. The effective dosages for human subjects were determined based on previous studies[34)38)-39)]. Accordingly, a dosage of 150 mg/day HCD (150H) and 300 mg/day HCD (300H) were selected for this investigation. Placebo capsules containing β-CD were used as a control. The HPTG content in the 150H and 300H capsules was 19.5 mg/day and 39.0 mg/day, respectively (see **Table 1**). Both the treatment and placebo capsules were indistinguishable in appearance.

2.3. 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



※HDL < 40 mg/dL: Japan Atherosclerosis Society Guidelines 2022[40] published high-density lipoprotein cholesterol (HDL) < 40 mg/dL as diagnostic criteria for dyslipidemia.
0H, Placebo; 150H, HCD 150 mg/day; 300H, HCD 300 mg/day.

Figure 1　Systematic flow chart of the study

were thoroughly explained. The inclusion criteria for participants selection were as follows: (1) Japanese males and females aged 30-64 years at the time of providing informed consent; (2) subjects who have no history of smoking within a year; (3) subjects who can record the electronic diary using smartphones or PCs; (4) BMI less than 30.0 kg/m^2; (5) subjects who received a sufficient explanation for the objective and summary of the trial, voluntarily decided to participate in the trial after understanding the purpose and making the written agreement. The following exclusion criteria were applied in this study: (1) subjects who have been currently visiting a hospital or treated with any drug or herbal remedy for any disease; (2) subjects who are on diet/exercise therapy under the guidance of a doctor; (3) subjects who currently have or have history of severe diseases; (4) subjects who daily use machines or take medicine, quasi-medicine, food for specified health uses or nutritional supplements/foods on the market that have health claims related to fatigue, stress relief, cold intolerance, blood pressure, glycemia, or fat; (5) subjects who daily have drinks and foods containing bellows (excluding individuals who can refrain from ingestion after receiving informed consent until the end of the study) - any part (extract, pulp, or peel) of citrus fruits such as mandarins, lemons, grapefruits and sudachis- any kinds of herb; (6) subjects who currently have or have a history of drug and/or food allergies; (7) subjects who are engaged in night work or spilt shift; (8) subjects who routinely take alcohol more than 60 g/day; (9)subjects who have unstable menstrual cycle or those who have the prospect to have menstruation on the test date; (10) subjects who have habits of daily exercise such as jogging and muscle training; (11) subjects who have plans of major change in their lifestyle (e.g., diet, sleep, and exercise) during the study period; (12) subjects who are planning to travel abroad during the study period; (13) subjects who are currently pregnant, breastfeeding, or planning to get pregnant during the study period; (14) subjects who joined other clinical trials within one month before receiving informed consent in this study, or those who are currently joining other clinical trials; (15) subjects who are judged by the physician to be unsuitable for this study.

2.4. Study design

The study was performed in a randomized, parallel, double-blind, and placebo-controlled manner, and a systematical flow chart of the study protocol is illustrated in **Figure 1**. Sixty subjects (n = 60) were selected from 124 subjects based on the inclusion/exclusion criteria. Throughout the course of the study, no dropouts occurred, and 60 subjects remained in the study for the safety analysis. However, one participant was

Table 2 Baseline characteristics of study subjects (n = 59)※

Parameter	0H	150H	300H	Total
Number	20	20	19	59
(Male/Female)	(12/8)	(10/10)	(9/10)	(31/28)
Age (years old)	45.5 ± 15.6	47.2 ± 13.3	45.4 ± 14.4	46.1 ± 14.2
Height (cm)	167 ± 9.43	166 ± 7.57	164 ± 8.16	165 ± 8.39
Body Weight (kg)	61.3 ± 13.20	59.9 ± 8.15	57.6 ± 10.50	59.6 ± 10.70
BMI (kg/m^2)	21.8 ± 2.92	21.7 ± 2.20	21.4 ± 2.20	21.6 ± 2.43
FMD (%)	4.3 ± 1.55	4.6 ± 1.67	4.6 ± 1.58	4.5 ± 1.58

※Values are means ± SD.; Key as illustrated in Table 1.

subsequently excluded from the efficacy analysis due to not meeting the diagnostic criteria (HDL <40 mg/dL) for dyslipidemia as outlined in the Japan Atherosclerosis Society Guidelines 2022[40] aligning with the study protocol. As a result, a total of 59 subjects were included in the validity analysis. The allocation of subjects was performed by IMEQRD Co., Ltd. (Tokyo, Japan), and the concerning information was concealed from both the subjects as well as the investigators until the completion of the intervention study.

2.5. Schedule and outcomes

The study was conducted at Higashi-Koganei-Sakura Clinic (Koganei City, Tokyo) from August 9th to November 27th, 2022. The participants were assigned to receive either 150 mg HCD, 300 mg HCD, or a placebo (2 capsules per day). The capsules were taken after breakfast and dinner for a duration of twelve weeks.

During the study period, participants were instructed to maintain their regular lifestyle and avoid taking pharmaceuticals (including external preparation), quasi-drugs, Chinese medicine, and functional food. If any of the aforementioned were taken, participants were required to record it in their daily logbook. Excessive exercise and alcohol consumption were also discouraged from the day prior to clinic visits. Participants were instructed to diligently record tablet intake, physical condition, use of pharmaceuticals, and any adverse events in the logbook. Throughout the 12-week study period, the following outcomes and safety evaluations were conducted.

The outcomes included the measurement of FMD, and the evaluations of weariness, mental stress, dark circles under the eyes, facial dullness, and swelling of the eyelids were selected on the basis of previous HSP-related studies[36)45)-49)]. These assessments were performed at baseline (0 w) and after 12 weeks of consumption (12 w).

Furthermore, safety evaluations were carried out through plasma biochemical tests, plasma hematological tests at 0 w and 12 w, as well as monitoring blood pressure, heart rate, weight, and BMI, as well as conducting medical interviews at 0 w, 4 w, 8 w, and 12 w. Participants also maintained a daily logbook throughout the study period.

2.6. Methods of evaluation of FMD

The evaluation of FMD was conducted by trained investigators using the UNEX EF18VG system (UNEX Corporation, Nagoya, Japan). FMD measurements were performed as follows: A longitudinal image of the brachial artery was captured before and after including a vascular response to reactive hyperemia through a 5-minute period of forearm occlusion, enabling the assessment of FMD levels. The baseline diameter was determined as the average vessel diameter during a 2-minute baseline period, while the peak diameter represented the largest diameter observed post-occlusion. FMD % was calculated as the percentage change from the baseline diameter. FMD % = ([peak diameter − baseline diameter]/baseline diameter) × 100[41].

2.7. Evaluation of quality of life (QOL)

QOL questionnaires on weariness, mental stress, dark circles under the eyes, facial dullness, and eyelid swelling were assessed using a VAS[42] at 0 w and 12 w. The VAS score represents the subjects' self-rated mental or physical state on a scale from 0 (worst) to 100 (best). Δ VAS was calculated as the change in VAS score from 0 to 12 weeks after the intake of 150H or 300H was compared to placebo (0H).

2.8. Statistical analysis

The statistical analysis was performed using R version 4.2.2., and the statistical significance evaluation of food (150H, or 300H group vs 0H group) was analyzed in a two-sided test at 12 weeks or in variation from 0 to 12 weeks using Dunnett's test (DNT) in the parametric, and Steel's test (STL) in the nonparametric for comparison for the placebo group (0H). Results with $^{**}p < 0.01$, $^{*}p < 0.05$ were considered statistically significant, and $^{\#}p < 0.1$ was nearly significant. The results are shown as mean ± standard deviation (SD).

Table 3　Evaluation of the efficacy of FMD among study subjects

Parameter	Group	N	FMD (%)	*p*-Value	Statistical analysis
FMD (%)	0H	20	4.76 ± 2.46		
	150H	20	5.37 ± 1.75	0.303	STL
	300H	19	6.46 ± 2.41	0.042*	STL

Values are means ± SD.
*$p < 0.05$ versus the placebo group; Steel's test (STL).

3. RESULT AND DISCUSSION

3.1. Characteristics of participants

The fifty-nine (n = 59, M: 31; F: 28) participants with plasma HDL ≧ 40 mg/dl in healthy adult males and females, were assigned to the 150H or the 300H or placebo (0H) supplementation groups through baseline characters (age, gender, height, body weight, BMI, and FMD (%)) (**Table 2**). There was no significant difference in any of the parameters between the groups at baseline.

3.2. Evaluation of FMD

The data obtained from the evaluation of FMD are summarized in Table 3. In subjects (n = 59), FMD (%) exhibited a concentration-dependent increase after 12 weeks of HCD intake (0H: 4.76 ± 2.46, 150H: 5.37 ± 1.75, and 300H: 6.46 ± 2.41). Statistical analysis using Steel's test (STL) in the clinical trial showed a significant difference ($p = 0.042$*) between the 300H and placebo (0H) groups, while the difference between the 150H and placebo (0H) groups was not significant ($p = 0.303$) (**Table 3, Figure 2**).

Corretti et al.[8] reported that the FMD technique induces the release of NO, which leads to vasodilation and can be quantitated as an index of vasomotor function. Additionally, Hosoo et al.[43] reported that increased FMD levels contribute to the smoothness and softness of blood vessels. These findings indicate that 12 weeks of HCD intake, specifically the 300H concentration, in healthy subject's results in enhanced smoothness and softness of blood vessels compared to the placebo group (0H). This effect helps maintain the elasticity of arteries, serving as a preventive measure against vascular diseases such as arteriosclerosis and cardiovascular diseases.

Buscemi et al.[23] observed a significant increase in FMD of 2.20% after 1.5 weeks of consuming 159.5 mg/day of HSP in 500 mL/day of orange juice. Rizza et al.[24] reported a 2.48% increase in FMD after 3 weeks of consuming 500 mg/day of HSP. Similarly, Salden et al.[25] demonstrated that individuals who consumed 450 mg/day of HSP for 6 weeks were protected from a decline in FMD following a high-fat meal. Furthermore, Valls et al.[44] reported a significant increase in ischemic reactive hyperemia, indicating improved endothelial-dependent vasomotor function, in subjects (n = 159) with pre- or stage 1 hypertension who consumed orange juice containing 600 mg/day of HSP for 12 weeks. Yari et al.[26] reported a significant reduction in systolic blood pressure (SBP) and improved endothelium-dependent vasodilation and potassium channel activity in subjects who consumed 1 g/day of HSP for 12 weeks. Based on these results, it is demonstrated that HSP intake (159.5 mg-1g/day, 1.5-12 weeks) shows promising efficacy in improving endothelial dysfunction.

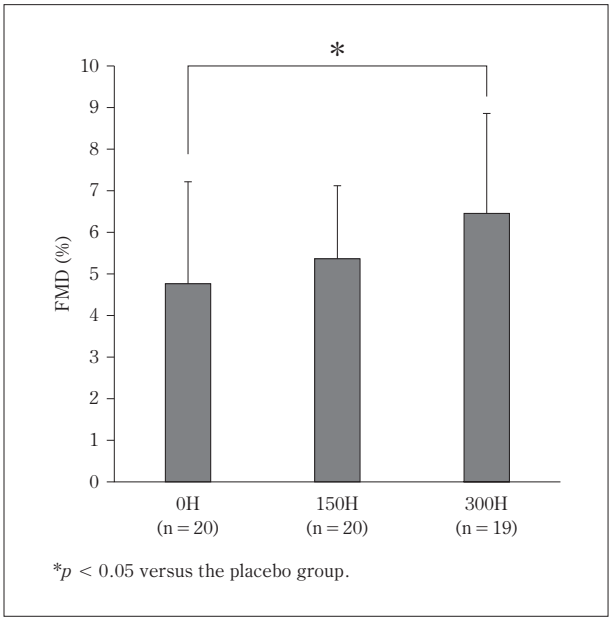


Figure 2　FMD (%) comparison in HCD intakes compared to placebo at 12 weeks

In this clinical study, we observed that the intake of 300 mg of HCD, which contained 39.0 mg of HPTG, led to improvements in FMD. These findings suggest that it is possible to enhance vascular endothelial function in healthy individuals with a lower dosage compared to the amount of HSP required for FMD improvement as reported in previous clinical studies. Notably, the concentration of total hesperetin (HPT), resulting from the breakdown of HPT metabolites by β -glucuronidase, was shown to be namely 100-fold higher in the bloodstream up to 24 hours after HCD intake compared to HSP intake, as demonstrated in a group of eight healthy subjects.[36] Therefore, this improved effectiveness

Table 4A Change in VAS score (ΔVAS) of mental or physical parameters among study subjects (n = 59)

Category	Group	N	Δ VAS (12-0 W)	*p*-Value	Statistical analysis
Weariness	0H	20	−3.88 ± 22.26		
	150H	20	9.18 ± 19.47	0.112	DNT
	300H	19	17.29 ± 23.33	0.007**	DNT
Mental stress	0H	20	−3.03 ± 26.89		
	150H	20	7.85 ± 19.34	0.283	DNT
	300H	19	9.58 ± 26.84	0.200	DNT
Dark circles under the eyes	0H	20	3.55 ± 17.22		
	150H	20	12.60 ± 18.75	0.326	DNT
	300H	19	20.47 ± 28.25	0.035*	DNT
Facial dullness	0H	20	5.25 ± 18.87		
	150H	20	13.45 ± 15.42	0.457	DNT
	300H	19	18.13 ± 34.17	0.175	DNT
Eyelid swelling	0H	20	3.68 ± 22.13		
	150H	20	6.15 ± 20.68	0.937	DNT
	300H	19	9.34 ± 33.92	0.723	DNT

**$p < 0.01$, *$p < 0.05$ versus the placebo group.
; Key as illustrated in Table 1; Steel's test (STL); Dunnett's test (DNT)

Table 4B Change in ΔVAS scores of the subjects with under median baseline scores

Category	Group	N	Δ VAS (under median baseline)	*p*-Value	Statistical analysis	Median
Weariness	0H	8	7.69 ± 23.13			
	150H	12	17.46 ± 17.00	0.496	DNT	60.5
	300H	10	28.05 ± 24.29	0.094 #	DNT	
Mental stress	0H	10	6.90 ± 23.61			
	150H	12	13.96 ± 20.22	0.703	DNT	61
	300H	8	30.38 ± 26.71	0.076 #	DNT	
Dark circles under the eyes	0H	6	17.42 ± 17.62			
	150H	13	17.23 ± 19.47	1.000	DNT	66
	300H	11	31.50 ± 31.24	0.397	DNT	
Facial dullness	0H	7	19.79 ± 20.59			
	150H	13	17.35 ± 14.97	0.889	STL	67.5
	300H	10	41.20 ± 30.27	0.099 #	STL	
Eyelid swelling	0H	8	11.31 ± 28.02			
	150H	14	7.39 ± 24.71	0.908	DNT	78.5
	300H	8	42.00 ± 21.35	0.036*	DNT	

**$p < 0.01$, *$p < 0.05$ versus the placebo group; #$p < 0.1$ versus the placebo group.
; Key as illustrated in Table 1; Steel's test (STL); Dunnett's test (DNT)

may be attributed to the high bioavailability of HCD.

3.3 Evaluation of Δ VAS scores

Table 4A shows Δ VAS scores, representing the change (12 w-0 w) of VAS scores in 5 categories including weariness, mental stress, dark circles under the eyes, facial dullness, and eyelid swelling, after supplementation with 150 mg and 300 mg HCD compared to the placebo (0H). The results demonstrate an HCD concentration-dependent improvement in each category (0H, 150H, and

300H) across the three groups. Notably, the 300H intake showed significant effectiveness in reducing weariness ($p = 0.007$**) and dark circles under the eyes ($p = 0.035$*), while the 150H group did not reach statistical significance ($p = 0.112$, $p = 0.326$, respectively). In addition, we conducted subgroup analysis by stratifying the scores of each evaluation item based on the median scores, as there were some subjects who had high scores (indicating little or no distress) on each evaluation item. **Table 4B** displays the results of ΔVAS scores for a subgroup of participants with baseline scores under the median in each respective category. This subgroup specifically evaluated individuals who had relatively poorer scores in those categories (eyelid swelling, mental stress, and facial dullness) at base line. The results reveal that the 300H group exhibited a significant improvement in scores for eyelid swelling compared to the placebo group ($p = 0.036$*). Furthermore, there was a tendency towards improvement in scores for weariness ($p = 0.094^{\#}$), mental stress ($p = 0.076^{\#}$) and facial dullness ($p = 0.099^{\#}$). These findings suggest that the intake of 300 mg of HCD may potentially alleviate eyelid swelling, mental stress, and facial dullness in individuals with more pronounced symptoms. However, no significant effect was observed in the 150H group across any of the evaluated categories.

Certain studies[45)-48)] have reported the efficacy of glucosyl hesperidin (G-HSP), which is also a water-soluble derivative of HSP in improving various health conditions. For instance, intake of G-HSP (400-500 mg/day), for one day to four weeks has shown the alleviation of swelling of the face and eye contours in the morning, swelling of the eye contours and dullness of the skin in the evening, and leg swelling[46)], morning awakening, low back pain, and dry skin[47)], as well as cold intolerance[48)] due to increasing NO production in vascular endothelial cells, enhanced blood flow, improving body fluid circulation and autonomic balance. Also, ingestion of G-HSP (50 mg/kg BW) for 8 weeks in rats prevented endothelial dysfunction and oxidative stress by reducing reactive oxygen species (ROS) properties, reducing urinary 8-hydroxy-2-deoxyguanosine (8-OHdG), an oxidative stress marker, and improving NO bioavailability in endothelial cells.

$AUC_{0\text{-}27}$ of total HPT glucuronide in plasma using HPLC after G-HSP ingestion in rats was only namely, 3.7-fold higher compared to HSP[49)], while HCD had greater bioavailability nearly 100-fold compared to HSP[36)]. Therefore, 300 mg HCD (contains 39 mg HPTG) could allow a lower dose than G-HSP, while resulting in potential alleviation of study parameters. The potential alleviation of weariness, mental stress, dark circles under the eyes, eyelid swelling, and facial dullness observed in this study might be attributed to the antioxidant properties and improvement of endothelial function brought about by HCD. The following mechanisms are considered: Firstly, the antioxidant capacity of HCD can suppress the generation of reactive oxygen species and inflammation, thereby improving the automatic nervous system, and potentially reducing weariness and mental stress. Additionally, by reducing oxidative damage and enhancing microcirculation, HCD may increase blood flow to the skin and subcutaneous tissues, thereby improving dark circles under the eyes and facial dullness. Furthermore, by improving body fluid circulation, HCD may alleviate eyelid swelling[45)-51)].

3.4 Safety

Some changes in Aspartate aminotransferase (AST) in 150H group and fasting blood sugar in 300H group were observed compared to the placebo in plasma at 12 weeks. However, these changes were within normal daily variation and were not considered to be related to the administration of test food. Therefore, it can be concluded that the intake of 150H and 300H (HPTG conversion: 19.5 mg and 39.0 mg, respectively) daily for twelve consecutive weeks as a test food did not cause any adverse effects in the present study.

4. CONCLUSION

The present study demonstrated that intervention with 300H for 12 weeks resulted in a significant improvement in endothelial dysfunction, as indicated by the increase in FMD (%). Furthermore, it showed positive effects in reducing weariness and dark circles under the eyes in healthy subjects. Additionally, the intake of 300H for 12 weeks led to a significant reduction in eyelid swelling in individuals with more pronounced symptoms. In terms of safety, no clinical abnormalities or adverse effects were observed during the 12-week consumption of 150H and 300H. These findings highlight the remarkable potential of HCD as a supplementary and functional food in promoting human health.

【Conflict of interest statement】

Taiyo Kagaku Co., Ltd. provided the funding for this study. There were no particular conflicts of interest. However, referring to a potential conflict of interest, MM, AA, MPK, AY, SO, and MO were employed by Taiyo Kagaku Co., Ltd.

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