Effects of Hydroxyhydroquinone-reduced Coffee in Patients with Essential Hypertension

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Recent animal and human studies suggest that chlorogenic acids, which are the main component of the class of polyphenols in coffee, reduce blood pressure, and that hydroxyhydroquinone (HHQ), produced by roasting green coffee beans, inhibits the antihypertensive effect of chlorogenic acids in brewed coffee. To examine the effects of 4 weeks of daily ingestion of HHQ-reduced coffee in patients with essential hypertension undergoing treatment with antihypertensive drugs. Patients being treated for essential hypertension with antihypertensive drugs participated in a randomized, double-blind, crossover controlled trial. After a 2-week run-in phase, participants consumed two cans of either HHQ-reduced coffee or Control coffee daily for 4 weeks. After a 2-week washout period, subjects were crossed over to the other treatment. Blood pressure and pulse rate were measured once a week. Blood biochemistry and hematology analysis was performed before and after the test beverage ingestion periods. A 4-week ingestion period of HHQ-reduced coffee did not significantly change systolic blood pressure (SBP) or diastolic blood pressure (DBP) compared to the Control coffee. There were no significant changes in pulse rate and body weight during the test beverage ingestion periods in either group, and no clinically relevant problems were reported. These findings suggest that 4 weeks of daily ingestion of HHQ-reduced coffee does not reduce or enhance the efficacy of antihypertensive drugs in treated essential hypertensive patients.

Key words —— coffee, hydroxyhydroquinone, blood pressure, hypertension

INTRODUCTION

Coffee is a widely ingested beverage that has been consumed for many centuries worldwide, and there are many studies of the effects of brewed coffee in association with human health. Coffee intake has protective effects against various diseases, such as type 2 diabetes,[1,2] liver dysfunction,[3,4] and Parkinson's disease.[5] The effects of the caffeine contained in coffee on cardiovascular disease, however, are controversial; many studies have reported negative effects, whereas others have reported no effect.[6] Many studies have examined whether coffee intake is involved in the elevation of blood pressure,[7–10] but as yet there is no evidence for its direct involvement.[11,12] Funatsu *et al.* recently reported that daily ingestion of brewed coffee from roasted coffee beans reduces blood pressure in humans who habitually drink alcoholic beverages.[13] The relationship between the ingestion of brewed coffee from roasted coffee beans and blood pressure is not clear.

Recent animal and human studies reported an antihypertensive effect of chlorogenic acids in green coffee bean extract, and the mechanism of action was suggested to be a nitric oxide (NO)-mediated improvement in vascular endothelial function.[14–16] Chlorogenic acids are compounds composed of caffeic acid or ferulic acid ester-bonded with quinic acid, and are found in green coffee beans at levels of 6.2% to 11.2%.[17] Chlorogenic acids mainly consist of 9 compounds: 5-caffeoylquinic acid as the most typical major component, and 3-caffeoylquinic acid, 4-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, 3-feruloylquinic acid, 4-feruloylquinic acid, and 5-feruloylquinic acid as minor components.[17] Although chlorogenic acids have beneficial effects on health, the antihypertensive effect and improved vascular endothelial func-

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tion induced by coffee brewed from roasted coffee beans is not well understood.

Suzuki *et al.* recently reported that hydroxyhydroquinone (HHQ) produced by roasting green coffee beans inhibits the antihypertensive effect of chlorogenic acids in brewed coffee. Further, an antihypertensive effect of brewed coffee was exhibited after reducing HHQ in an animal study using spontaneously hypertensive rats (SHR).[18] HHQ produces reactive oxygen species,[19] and reactive oxygen species are assumed to reduce the antihypertensive effect of chlorogenic acids. Furthermore, Chikama *et al.* reported that 12 weeks of continuous ingestion of the chlorogenic acids contained in HHQ-reduced coffee decreased blood pressure and improved vascular endothelial function in untreated mildly hypertensive subjects.[20–23] Based on these findings, HHQ-reduced coffee might have great public health benefits.

HHQ-reduced coffee has an antihypertensive effect in untreated mild hypertensive subjects. Its mechanism of action is different from that of most antihypertensive drugs. Thus, it would be very important to estimate the effect of antihypertensive drugs in patients ingesting HHQ-reduced coffee. In this study, we investigated the effects of HHQ-reduced coffee in patients with essential hypertension undergoing antihypertensive drug treatment.

MATERIALS AND METHODS

Subjects —— The subjects were 32 male and female with hypertension, aged 35 to 70 years upon initiation of the study. The subjects were recruited from a metal foundry (Chiba, Japan). All subjects had taken antihypertensive drugs for at least 1 month, and had stable blood pressure. Subjects who met the following criteria were excluded from the study: severe liver, cardiovascular, or endocrine disorders; pregnant or planning to become pregnant; heavy cigarette smoking habit (mean > 20 cigarettes/day); heavy alcohol drinking habit (mean > 50 g/day); allergy to caffeine or coffee; or ineligible as judged by the physician in charge. The study was approved by the Mitsukoshi Health and Welfare Foundation Research Committee, and performed at the medical office of the metal foundry. All subjects provided written informed consent. The clinical trial was performed with adequate consideration under the supervision of the principal investigator in accordance with the Helsinki Declaration (1964) and its revised version (2002).

Test Beverages —— Normal brewed coffee contains both chlorogenic acids and HHQ. Based on high performance liquid chromatography analysis, a cup of coffee typically contains 40 to 350 mg of chlorogenic acids and 0.1 to 1.7 mg of HHQ (data not shown). The HHQ-reduced beverage was prepared by reducing the HHQ content of a commonly consumed, commercially available coffee product using the adsorption method; the control beverage contained HHQ, and was the same commonly consumed coffee. Both the HHQ-reduced and Control beverages contained chlorogenic acids. The test beverages were prepared using a popular Japanese canned coffee drink (184 ml) and could not be distinguished from each other in appearance or taste. The HHQ-reduced and control beverages contained (per 184 ml) 299 mg chlorogenic acids and, 0.05 and 1.69 mg HHQ, 77 and 75 mg caffeine, and 29.3 and 37.7 kJ, respectively.[23]

The Study Protocol —— The study design was a randomized, double-blind, crossover controlled trial (Fig. 1). Blood pressure and pulse rate were measured once a week during the 2-week run-in period before ingestion of the test beverage. At the end of the run-in period, both groups were randomly assigned to consume either 2 cans of HHQ-reduced beverage/day for 4 weeks, a 2-week washout, 2 cans of Control beverage/day for 4 weeks, and a 2-week washout; or 2 cans of Control beverage/day for 4 weeks, a 2-week washout, 2 cans of HHQ-reduced beverage/day for 4 weeks, and a 2-week washout. The first 4-week ingestion period was called Period 1, and the second ingestion period was called Period 2. Blood pressure and pulse rate were measured once a week during the test beverage ingestion periods, and blood analysis was performed before and after the test beverage ingestion periods. The subjects were instructed to continue taking antihypertensive drugs and other treatments during the study period; to continue their usual eating, exercise, and cigarette-smoking habits; and to refrain from overeating and overdrinking. Ingestion of drugs that affect blood pressure, excluding those prescribed by a physician, and foods that improve hypertension were prohibited. Ingestion of coffee other than the test beverages was prohibited during the test beverage ingestion periods. On blood pressure measurement days, the subjects were instructed to ingest antihypertensive drugs as usual, and drink the test beverage at least 2 hr before the measure-

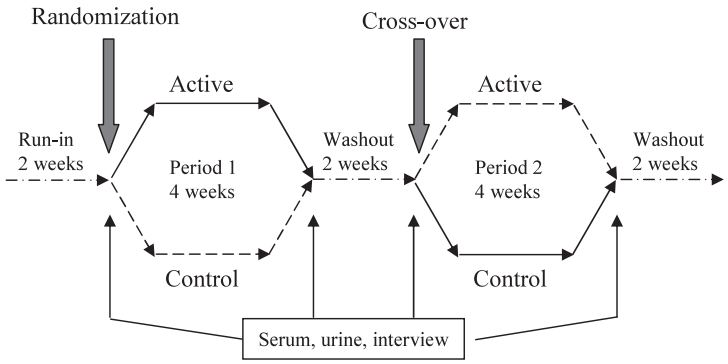

Fig. 1. Study Design

ment. On the day prior to collection, the subjects were instructed to finish eating dinner by 21:00, and to not drink or eat anything other than water thereafter until completion of the measurements. The measurement items were systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse rate, height, body weight, hematology, blood chemistry, urinalysis, and interview.

Measurement of Blood Pressure and Pulse Rate and Interview —— Blood pressure and pulse rate were measured at least two times with intervals of at least 1 min by a nurse using an automatic sphygmomanometer after the subjects visited the office in the morning and had rested in a sitting position for at least 5 min. The mean of two similar values (difference between the measured values less than 5 mmHg) was recorded. For the interview, a physician questioned the subjects regarding the presence or absence of subjective symptoms and examined them for objective findings.

Hematology, Blood Chemistry, and Urinalysis —— Hematology, blood chemistry, and urinalysis studies were performed before and after the test beverage ingestion periods. On test days, a skilled nurse collected blood after the subject had rested in a sitting position for at least 10 min. The hematology items were red and white blood cell counts, hemoglobin, hematocrit, and platelet count. The blood chemistry items were triglycerides, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, fasting blood glucose, total homocysteine, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, alkaline phosphatase, lactate dehydrogenase, total protein, albumin, albumin-globulin ratio, bilirubin, uric acid, urea nitrogen, creatinine, sodium (Na), inorganic phosphorus, potassium (K), calcium (Ca), magnesium (Mg), iron, unsaturated iron-binding capacity, ferritin, high-sensitive C-reactive protein (hsCRP), plasminogen activating factor-1 (tPAI-1), interleukin 6 (IL-6), and 8-isoprostane. Urinalysis was performed using test papers. The test items were pH, glucose, occult blood, urobilinogen, and ketone bodies. Hematology was performed by Sanritsu Co. (Chiba, Japan) and SRL Inc. (Tokyo, Japan).

Height and Body Weight —— Height was measured upon initiation of the run-in period before test beverage ingestion. Body weight was measured upon initiation of the run-in period and after each test beverage ingestion period.

Statistical Analysis —— The primary endpoint was variation in SBP and DBP after continuous ingestion of the HHQ-reduced beverage for 4 weeks in subjects with essential hypertension undergoing antihypertensive drug treatment. The full analysis data set was defined as the largest analyzable population, excluding subjects who underwent testing after randomization, consistent with the intention-to treat principle.

To evaluate variations in SBP and DBP, a linear mixed-model analysis was used to assess the significance of changes in the actual values at 0, 1, 2, 3, and 4 weeks, with Group, week, Group*week interaction as fixed effects. For the evaluation of hematology, blood chemistry, urinalysis, weight and pulse rate, a comparison between the groups at week 4 in Period 1 was performed using Student's t-test. For all items, group differences at week 0 were analyzed using Student's t-test.

The data are presented as the mean ± standard error of mean (SEM). All tests were two-sided, and

Table 1. Baseline Characteristics

Variable	Males (n = 27) Mean (SEM) (min, max)	Females (n = 4) Mean (SEM) (min, max)
Ethnicity	Japanese	Japanese
Age (years)	55.1 (1.0) (39 , 64)	58.8 (3.9) (49 , 66)
Height (cm)	166.7 (0.8) (157.0, 174.5)	156.8 (1.6) (152.5, 160.0)
Weight (kg)	67.6 (1.5) (50.5, 87.0)	60.5 (5.8) (45.0, 73.0)
SBP (mmHg)	137.1 (2.5) (122.0, 166.5)	137.6 (7.6) (119.0, 153.5)
DBP (mmHg)	85.5 (1.4) (71.0, 97.0)	79.1 (4.0) (72.0, 90.0)

$p < 0.05$ was regarded as significant. Statistical calculations were performed using SPSS 15.0J for windows (SPSS Japan Inc., Tokyo, Japan).

RESULTS

Subject Characteristics

Thirty-two hypertensive subjects undergoing antihypertensive drug treatment were judged eligible for the study and provided written informed consent and enrolled in the study. The enrolled subjects were randomly allocated to the treatment groups. One female subject in the HHQ-reduced beverage group dropped out during the course of the study because of a stomachache during Week 1 of Period 1. Thus, 31 subjects were included in the analyses. The baseline characteristics of the 31 analyzed subjects are shown in Table 1, and their antihypertensive drug prescriptions are shown in Table 2.

Twenty-seven male and 4 female hypertensive subjects were analyzed. Although 11 subjects were being treated with antihypertensive drugs, their SBP was still classified as hypertensive (SBP; ≥140 mmHg).[24] Nine subjects were treated with a single drug: a Ca antagonist ($n = 3$), an angiotensin converting enzyme inhibitor (ACEI) ($n = 3$), or an angiotensin II receptor blocker (ARB) ($n = 3$). Twenty-two subjects were treated with combination therapy; 16 were treated with 2 drugs (a Ca antagonist combined with an ACEI or ARB), and 6 subjects were treated with 3 or more drugs (antihypertensive drugs with other action mechanisms were combined with the Ca antagonist and ACEI or ARB). The following drugs were administered; Ca antagonists: amlodipine, nifedipine, and felodipine; ACEI: imidapril hydrochloride and trandolapril; ARB: valsartan; diuretic: trichlormethiazide; α-blocker: bunazosin hydrochloride; and β-blockers: atenolol and carteolol hydrochloride. Of the 31 subjects, 10 were complicated by other diseases (hyperlipidemia in 5, diabetes in 2, gout in 1, and gastritis in 2), and undergoing drug treatment for various symptoms. The prescribed drugs were not modified for any of the subjects during the study period.

Table 2. Antihypertensive Drugs Prescribed

Antihypertensive Drugs	Cases [percentage (%)]
CCB	3 [9.7]
ACEI	3 [9.7]
ARB	3 [9.7]
CCB + ACEI	6 [19.4]
CCB + ARB	8 [25.8]
CCB + BB	1 [3.2]
ACEI + AB	1 [3.2]
CCB + ACEI + ARB	4 [12.9]
CCB + ACEI + BB	1 [3.2]
CCB + ARB + AB + diuretic	1 [3.2]
Total	31 [100]

CCB, calcium channel blocker; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BB, β-blocker; AB, α-blocker.

Changes in SBP and DBP

There was a significant difference in SBP prior to the test beverage ingestion between Period 1 and Period 2 (137.2 ± 2.4 *vs.* 131.6 ± 2.6 mmHg; $p < 0.001$). These findings suggested a carryover effect, and the carryover effect was noted in both the HHQ-reduced and Control Period 1 beverage group. Changes in SBP and DBP during the test beverage ingestion periods are shown in Fig. 2a and 2b. There was no significant difference between the HHQ-reduced and Control groups. In an analysis of baseline SBP ≥ 140 mmHg subjects, changes in blood pressure were not significant between groups. Changes in SBP and DBP during the test beverage ingestion period were analyzed in patients treated with 1 ($n = 9$), 2 ($n = 16$), and 3 or more ($n = 6$) antihypertensive drugs, but there were no significant differences between the HHQ-reduced and Control groups (data not shown).

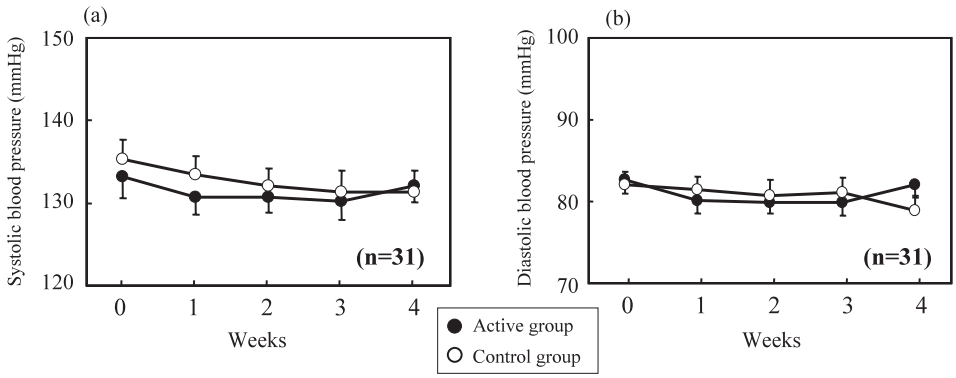

Fig. 2. Changes in Blood Pressure in Hypertensive Patients (●; Active group, ○; Control group)
(a) Systolic blood pressure or (b) Diastolic blood pressure. Each value represents the mean ± S.E., n = 31. No significant differences were found between the Active and Control groups by a linear mixed-model analysis.

Changes in Body Weight, Pulse Rate, Hematology, Blood Chemistry, and Urine Test

Table 3 shows body weight, pulse rate, hematology, and blood chemistry after Period 1. Aspartate aminotransferase was significantly different between the HHQ-reduced and Control groups. There were no significant differences in the changes between the HHQ-reduced and Control groups for any other item. No clinically remarkable findings were noted in any qualitative urinalysis item: pH, protein, glucose, occult blood, urobilinogen, and specific gravity (data not shown).

Adverse Events

No adverse events related to blood pressure attributable to the ingestion of chlorogenic acid were noted in interviews by physicians. There were no clinically problematic changes in laboratory test values in individual subjects.

DISCUSSION

In the present study, we investigated the effects of HHQ-reduced coffee on blood pressure in hypertensive subjects undergoing antihypertensive drug treatment. These hypertensive subjects continuously ingested the test beverages, and the effects on blood pressure during the ingestion periods were evaluated. One female subject in the HHQ-reduced beverage group reported a stomachache and dropped out 1 week after initiation of the test beverage ingestion during Period 1, but no problems were noted on interview or upon examination by a physician. Based on the interview, her stomachache might have been due to drinking 2 cans of black coffee daily, because this subject did not usually drink black coffee so frequently.

In this study, there was a carryover effect of SBP prior to the test beverage ingestion in the time Period 2. Blood pressure taken during the washout period was reduced from that prior to Period 1 in both groups. At the end of the washout period, however, SBP values remained lower than that prior to the test beverage ingestion of Period 1. These findings suggest that the washout period was not long enough to wash out all of the effects of ingesting the HHQ-reduced and Control beverages.

The 4-week ingestion of the HHQ-reduced and Control beverages tended to reduce blood pressure, and the HHQ-reduced beverage did not significantly change SBP or DBP compared to the Control group. These findings suggest that ingestion of HHQ-reduced coffee does not reduce or enhance the efficacy of antihypertensive drugs in treated essential hypertensive patients.

The aspartate aminotransferase levels were significantly different between the two groups. The aspartate aminotransferase levels, however, were within the normal range. There were no significant changes in either group in pulse rate, body weight, hematology, and blood chemistry items except for aspartate aminotransferase, during the test beverage ingestion periods, and no clinically problematic changes. These finding suggest that the reductions were not specific to HHQ-reduced coffee, and were due to changes in life-style brought about by the study. The chlorogenic acids did not induce any adverse effects that were detected by physician-conducted interviews.

HHQ-reduced coffee ingestion is reported to reduce blood pressure in patients with mild hyper-

Table 3. Changes in Weight, Pulse Rate and Blood Chemistry Parameters

Variables	Active $n = 16$	Control $n = 15$	p
Weight (kg)	63.3 (1.8)	68.2 (2.3)	0.115
Pulse rate (bpm)	76.2 (2.0)	80.4 (3.7)	0.334
Triglyceride (mg/dl)	155.0 (22.5)	113.3 (16.5)	0.143
Total-cholesterol (mg/dl)	208.9 (7.8)	214.2 (6.3)	0.602
LDL-cholesterol (mg/dl)	118.8 (6.2)	128.3 (4.1)	0.209
HDL-cholesterol (mg/dl)	67.7 (4.4)	70.6 (3.8)	0.616
Total homocysteine (nmol/ml)	17.42 (1.34)	15.34 (0.67)	0.168
AST (IU/l)	21.4 (0.9)	25.7 (1.8)	0.046
ALT (IU/l)	19.9 (2.0)	25.0 (2.8)	0.151
γ-GTP (IU/l)	38.3 (6.2)	49.6 (9.2)	0.323
White blood cell (10^3/μl)	6.35 (0.54)	5.86 (0.44)	0.491
Red blood cell (10^4/μl)	475.5 (8.7)	478.3 (11.3)	0.845
Hemoglobin (g/dl)	14.56 (0.26)	14.69 (0.30)	0.737
Hematocrit (%)	46.07 (0.82)	46.36 (1.00)	0.830
Platelet (10^4/μl)	25.19 (1.59)	23.77 (1.97)	0.581
Total Protein (g/dl)	7.39 (0.12)	7.43 (0.09)	0.796
Albumin (g/dl)	4.55 (0.08)	4.58 (0.05)	0.826
ALP (IU/l)	220.9 (15.8)	247.3 (30.1)	0.453
LDH (IU/l)	189.8 (5.8)	202.8 (7.3)	0.176
Uric acid (mg/dl)	6.57 (0.55)	5.93 (0.34)	0.325
BUN (mg/dl)	14.69 (0.84)	15.06 (0.84)	0.759
Creatinine (mg/dl)	0.893 (0.044)	0.833 (0.059)	0.431
Fasting blood sugar (mg/dl)	98.1 (3.5)	109.7 (8.8)	0.242
Albumin-globulin ratio (%)	1.61 (0.04)	1.61 (0.04)	0.983
Bilirubin (mg/dl)	0.78 (0.12)	0.76 (0.07)	0.901
Na (mEq/l)	141.8 (0.5)	141.8 (0.5)	0.942
Cl (mEq/l)	102.2 (0.6)	101.6 (0.6)	0.433
K (mEq/l)	4.33 (0.06)	4.35 (0.11)	0.855
Ca (mg/dl)	9.43 (0.09)	9.45 (0.07)	0.832
Inorganic phosphorus (mg/dl)	3.34 (0.14)	3.18 (0.13)	0.419
Mg (mg/dl)	2.27 (0.03)	2.23 (0.04)	0.332
Ferritin (ng/ml)	116.7 (25.4)	161.5 (22.3)	0.193
Fe (μg/dl)	102.1 (10.3)	112.8 (7.9)	0.414
UIBC (μg/dl)	247.8 (16.7)	220.5 (10.7)	0.174
hsCRP (ng/ml)	547.7 (147.1)	669.1 (211.0)	0.645
tPAI-1 (ng/ml)	21.9 (3.7)	27.3 (3.8)	0.319
IL-6 (pg/ml)	1.38 (0.39)	0.82 (0.10)	0.163
isoprostane (pg/ml)	11.3 (0.4)	11.3 (0.4)	0.901

Mean (S.E.). Abbreviation: p, p-value; LDL, Low density lipoprotein; HDL, High density lipoprotein; AST, Aspartate aminotransferase ALT, Alanine-amino transpeptidase; γ-GTP, γ-glutamyl transpeptidase; ALP, Alkaline phosphatase; LDH, Lactate dehydrogenase; BUN, Blood urea nitrogen; UIBC, Unsaturated iron-binding capacity; hsCRP, high-sensitive C-reactive protein; tPAI-1, plasminogen activating factor-1; IL-6, interleukin 6; isoprostane, 8-isoprostane. p is p-value of group effect by the Student's t-test.

tension and high-normal blood pressure,[20,21] and chlorogenic acids might be the antihypertensive component. In an animal study using a concomitant NO synthesis inhibitor, N(G)-nitro-L-arginine methyl ester, in SHR, Suzuki *et al.* reported that ingestion of the main component of chlorogenic acids, 5-caffeoylquinic acid, reduces blood pressure along with the production of NO in the vascu-

lar endothelium[25] and reduces NADPH-dependent superoxide anion production in isolated SHR thoracic aorta, suggesting that chlorogenic acids improve the vascular endothelium-dependent vasodilatation reaction. Blood pressure might be lowered, at least partially, by the following mechanism: chlorogenic acids inhibit excess reactive oxygen species production and increase NO bioavailability, which improves vascular endothelial function and reduces blood pressure. In clinical trials, green coffee bean extract (the main component of which is chlorogenic acids) improved vascular endothelial function.[26] These findings suggest that HHQ-reduced coffee decreases blood pressure by improving vascular endothelial function. In the present study, more than 80% of the subjects were treated with either an ACEI or ARB. In addition to lowering blood pressure, these drugs improve vascular endothelial function through NO.[27–29] Thus, blood pressure changes were analyzed in relation to the presence or absence of ACEI and ARB treatment, but no significant changes were noted in SBP or DBP in the HHQ-reduced group compared to the Control group. These findings support the idea that HHQ-reduced coffee reduces blood pressure by improving vascular endothelial function, but it might be not reduce or enhance the efficacy of antihypertensive drugs in treated essential hypertensive patients.

In conclusions, a crossover study of HHQ-reduced coffee containing chlorogenic acids, 299 mg in can was performed. Thirty-one subjects undergoing antihypertensive drug treatment ingested 2 cans of HHQ-reduced coffee daily for 4 week. No significant changes were noted in blood pressure in the HHQ-reduced or Control groups. In terms of safety, no adverse events attributable to the ingestion of chlorogenic acids were noted. Based on these findings, four weeks of daily ingestion of HHQ-reduced coffee containing chlorogenic acids does not reduce or enhance the efficacy of antihypertensive drug in treated essential hypertensive patients.

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