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Effects of Green Coffee Extract Supplementation on Oxidative Stress, Systemic and Vascular Inflammation in Patients with Metabolic Syndrome: A Randomized Clinical Trial

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Abstract

Background: Metabolic syndrome (Mets) is accompanied by oxidative stress and low-grade inflammation. Green coffee is rich in polyphenols called chlorogenic acids (CGA), which possess anti-inflammatory and anti-oxidative characteristics.
Objectives: The aim of this study was to evaluate the effects of green coffee extract (GCE) on the oxidative stress as well as the systemic and vascular inflammation in patients having Mets.
Methods: This randomized clinical trial was conducted in 2016 in Iran. Forty-three individuals (21 in the intervention and 22 in the control group) with Mets were randomly assigned to take 400 mg GCE supplements twice a day in the intervention group or placebo capsules in the control group for 8 -weeks. The serum levels of intercellular adhesion molecule-1 (ICAM-1), interleukin-6 (IL-6), high sensitivity C-reactive protein (hs-CRP), and malondialdehyde (MDA) were evaluated at the beginning and 8 -weeks after the intervention.
Results: No significant discrepancy was observed regarding serum levels of IL-6, MDA, hs-CRP, and ICAM-1 between the intervention and control group at the beginning and the end of the trial. After eight weeks of intervention, the mean changes of IL6 in the treatment and the placebo group were respectively (-0.73 $\pm$ 2.65 VS 1.70 $\pm$ 10.51 Pg/mL, P value = 0.3), hs-CRP (-0.28 $\pm$ 3.12 VS -0.08 $\pm$ 4.15mg/L, P value = 0.86), MDA (0.44 $\pm$ 1.68 VS 0.32 $\pm$ 2.28 μmol/L, P value = 0.84), and ICAM-1 (-0.05 $\pm$ 0.45 VS 0.02 $\pm$ 0.45ng/mL, P value = 0.54).
Conclusions: In this trial, the green coffee extract (GCE) administration did not affect oxidative stress, systemic, and vascular inflammation in subjects with metabolic syndrome.

Keywords: Chlorogenic Acid, Inflammation, Metabolic Syndrome, Oxidative Stress

1. Background

Metabolic syndrome (Mets) is defined as a metabolic disorder that increases the risk for development of Type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (1). It is a prevalent metabolic disorder worldwide affecting around 20% to 25% of the world's adult population (2). Various factors such as abdominal obesity, insulin resistance, and pro-inflammatory state are involved in Mets etiology (2). Abdominal adiposity, as one of Mets features, induces inflammation and oxidative stress, which can both lead to the development of other Mets components such as hyperlipidemia, hypertension, and insulin resistance (3). Mets is accompanied by a low-grade inflammation due to hypertrophy of adipocytes, which results in a higher secretion of adipokines such as pro-inflammatory cytokines (4). Furthermore, another underlying cause of increased inflammation in the Mets is oxidative stress (OS). Increased fat mass in Mets causes lack of oxygen and cell necrosis. Phagocytosis of these dead cells produces free radicals such as nitric oxide and hydrogen peroxide aside from inflammatory compounds (3). OS is caused by lack of balance between antioxidants and oxidants (free radicals) such as reactive oxygen species (ROS) (3). The reaction of ROS with unsaturated lipids generates lipid peroxidation products such as malondialdehyde (MDA) (5). Both oxidative stress and inflammation in Mets result in damaging cells such as

endothelium, which leads to a vascular pro-inflammatory state, which is identified by increased expression of adhesion molecules by endothelial cells (6).

Coffee is a rich dietary source of antioxidants due to its high content of polyphenols called chlorogenic acids, and its consumption has been associated with the increased antioxidant capacity of plasma (7). Polyphenols are the most widely distributed antioxidants in our diet (8). Chlorogenic acids are esters of cinnamic acids with quinic acid, which are found in many plants (9). Green coffee beans are the main sources of chlorogenic acids (CGAs), since a large amount of CGAs are lost through the roasting process of coffee (10). The most abundant ester of CGAs in the green coffee bean is 5-caffeoylquinic acid (11). It has been indicated that CGAs possess antioxidative, anti-inflammatory, anti-obesity, anti-diabetes, anti-lipidemic, and anti-hypertensive characteristics (12). Several studies have exhibited that CGAs can reduce oxidative stress, which is evidenced by reducing malondialdehyde (13, 14) or oxidized low-density lipoprotein (ox-LDL) concentrations (13, 15) and increasing total antioxidant capacity (16) of plasma (17). In addition, in some studies it has been capable of decreasing inflammation by reducing tumor necrosis factor alpha (TNF-α), Interleukin-6 (IL-6) (18), Interleukin-1beta and Interleukin-8 (19) in animals. In addition, there is some evidence that CGAs can reduce vascular inflammation by inhibiting expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial cell selectins such as E-selectin and P-selectin (20-22).

To be more specific, one study investigating the effects of green and roasted coffee in diabetic rats demonstrated a significant reduction of plasma MDA levels after supplementation with green and light roasted coffee compared to the diabetic control group. In addition, the activity of plasma antioxidant enzymes such as superoxide dismutase and glutathione levels were increased (23). Furthermore, 0.3% green coffee extract plus a high- fat diet decreased inflammatory markers of IL-6 and monocyte chemoattractant protein-1 in mice in relation to the HFD group (11). However, one study on Mets model of mice indicated no improvement in systemic oxidative stress assessed by plasma F2-isoprostane with supplementation of 0.5% w/w GCE plus HFD for 12 weeks compared to HFD ingested group (24). Also, in one study, a very high dose of CGA (7 mg/kg) in rats could cause an elevation of IL-6 and TNF-α and MDA after seven days compared to the control group; however, the low dose (0.3 mg/kg) of CGA did not affect these biomarkers (25). Most of the previous researches are animal studies, which investigated CGA effects rather than GCE impact. In addition, their findings are rather inconsistent.

2. Objectives

Due to the fact that human clinical trials in this field are rare, and does not exit any data as well as the fact that clinical trials in animal are inconsistent for this objective, we conducted this randomized, double-blind, placebo-controlled trial to investigate GCE effects on inflammation and oxidative stress status in Mets patients.

3. Methods

3.1. Patients

This study is a randomized, double-blind, placebo-controlled trial. Men and women aged 18 to 70, who had Mets and body mass index of over 25 were selected from Imam Hossein Hospital Diabetes Clinic patients in Tehran, Iran, in 2016. In this study, our primary outcomes were inflammatory biomarkers.

To calculate the sample size, suggested formula for parallel randomized controlled trial was used based on 0.05 for type one (α) error and 0.20 for type two error (β) (power = 80%). According to the study of Ebrahimi et al. (26); We considered 1.02 mg/dL as standard deviation (SD) and 1.4 mg/dL as the difference in mean of high sensitivity C-reactive protein (hs-CRP) as the principal outcome variable. Therefore, we needed 20 subjects in each group. However, 25 patients were recruited per group to take into account the possible dropouts. Diagnosis of Mets was based on the new definition of Mets by International Diabetes Federation (IDF) (27), which is having abdominal obesity (waist circumference > 102 cm in men or > 88 cm in women) added to two of these factors: fasting blood glucose > 100 mg/dL, triglycerides (TG) > 150 mg/dL, high density lipoprotein cholesterol (HDL-C) < 50 in women or < 40 in men, systolic blood pressure (SBP) > 130 mmHg, and diastolic blood pressure (DBP) > 85 mmHg. The exclusion criteria include: Insulin administration for diabetes control, having hypo- or hyperthyroidism, renal failure or other chronic diseases, regular coffee consumption, pregnancy, breastfeeding, taking estrogen, progesterone, corticosteroids, weight loss supplements, and obeying unusual weight loss plans. In addition, the subjects in our study, who had not taken more than 10% of their GCE supplements, were excluded. This study was confirmed by the

Ethics Committee of National Nutrition and Food Technology Research Institute (NNFTRI) of Shahid Beheshti University of Medical Sciences, Tehran, Iran, and was registered at Clinical Trials.gov (ID: NCT03265184).

3.2. Sample Preparation

The study protocol was explained for the patients having our inclusion criteria, and the volunteer subjects signed a written informed consent. The patients were inquired about their smoking status, drug history, illnesses, and the duration of suffering from diabetes (for diabetic patients). Gender was stratified for subjects and randomly assigned to GCE or placebo group by use of stratified blocked randomization method, this was done with block sizes of four concealed in a container by one of the researchers and the other investigator randomly enrolled the participants to the GCE or placebo group.

3.3. Physical Activity, Dietary Intake, Anthropometric and Biochemical Assessments

We had only one observer for data collection in all sections of the study. Dietary intake was assessed at baseline, middle of trial, and at the end of the study using a three-day food record. The participants were instructed on how to record their food and beverage intake for three days at each time. In order to distinguish the accurate portion sizes, the patients were interviewed to report their intake based on household measures. After converting portion sizes to grams, Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods was used to assess energy and nutrient content of foods (28) and physical activity of patients was measured by the metabolic equivalent of task (29). All measurements were taken based on standard protocols. Height was measured in a standing position without shoes and by use of a tape measure stuck to the wall while the shoulders were in a normal state to the nearest 0.5 cm. Weight was measured with the subject being minimally clothed and without shoes by Seca digital scale to the nearest 100 grams. In addition, waist circumference was measured to the nearest 0.5 cm at the level of navel between the last rib and top of the iliac crest using a tape measure.

After fasting for 12 hours, 10 mL of venous blood was taken from the participants of both groups at baseline and at the end of the study. Blood samples were centrifuged at a rate of 2000 rounds per minute to separate their serums. Blood serums were distributed into microtubes and subsequently, they were stored at 80°C until the time of performing the experiments. Oxidative stress and systemic or vascular inflammatory biomarkers were measured before and after the intervention. Commercials kits were used to measure MDA, hs-CRP concentrations (Zellbio, Germany), and for measurement of IL-6, ICAM-1 concentrations (Diaclone, France). All the experiments were performed using the ELISA method.

3.4. Intervention

Patients and researchers were blind to the group assignments. Due to the fact that this was a blinded trial, the containers of supplements were coded with A and B by the manufacturer. Participants were requested to take 400 mg GCE capsules or placebo two times a day with their main meals for eight weeks. At the time of randomization, a bottle of supplements containing 64 capsules, adequate for 32 days, was given to both groups and the another bottle was given at the fourth week for follow up the patients. In addition, all of the patients who received a dietary plan with weight loss recommendations were given to patients in both groups. The diet included a macronutrient composition of 52% carbohydrates, 18% protein, and 30% total fat. All of the participants were taught not to alter their physical activity. Follow-up of the patients was performed by calling them every 15 days to make sure that they consumed the supplements. In addition, in the middle of the study, at the fourth week, a follow-up appointment was made for all of the subjects.

The decaffeinated green coffee extract supplements and placebo capsules were supplied by Arjuna Natural Extracts Ltd. (Arjuna Natural, Kerala, India). GCE capsules contained 46.45% total CGA, which was measured by high- performance liquid chromatography (HPLC) and green coffee bean material to extract ratio was 6: 1. Therefore, each GCE capsule was 400 mg, which was equivalent to 2400 mg green coffee bean and 186 mg of chlorogenic acids. 3-caffeoylquinic acid and 5-caffeoylquinic acid composed about 10% - 15% and 35% - 40% of the total chlorogenic acids, respectively. The total polyphenols in the GCE capsules were 53.8%, which was assayed by spectrophotometer. For preparing cholorogenic acids, extract used from alcohol and removing caffeine from extract Chloroform has been used. The stages for preparation of the extract were as follows, first water and powdered green coffee bean were combined at the temperature of 45°C - 55°C for 4 hours. Then, alcohol added to the mixture at 50°C - 55°C until the extraction is finished. Next, the solvent layer is dried and removed to gain a dry substance, which is later purified by dissolving in water and using chloroform for eliminating caffeine. After that, chloroform disserved from the

mixture and the aqueous portion was filtered and vaporized by spray drying to produce the dry powder. Placebo capsules contain starch without any additives.

3.5. Statistical Analyses

Data were analyzed and reported using IBM SPSS Statistics Software for Windows, version 21.0 (IBM Corp., Armonk, N.Y., USA). We used the Kolmogorov-Smirnov test to determine the normality of the distribution of the data. In order to compare qualitative variables between the groups, Chi-square test was performed. The differences between post- and pre- trial values in each group were compared by paired *t*-test. Furthermore, between groups, comparisons were made by Independent *t*-test. In addition, repeated measures ANOVA test was performed to compare values of dietary intake at baseline, at the fourth week and at the end of study for each subject. All repeated measurement assumptions (normality, sphericity, randomness, ...) were checked. P value of < 0.05 was set as the significance level, and the quantitative variables are all shown as the mean $\pm$ standard deviation (SD).

4. Results

Out of 50 individuals who were enrolled in the study (25 in intervention and 25 in the control group), 43 patients completed the trial. The rationale behind the exclusion of four subjects in the GCE group was not taking the capsules or commencement of insulin administration for controlling blood glucose. Moreover, three individuals were eliminated from the control group by reason of not consuming the placebo capsules. Table 1 indicates that the baseline qualitative characteristics of participants consisting of gender, smoking status, and medications used for controlling hyperglycemia, hypertension, and lipid profile did not significantly differ between the groups. Likewise, baseline quantitative characteristics including age and duration of suffering from diabetes were not different between the intervention and control group (Table 2). Additionally, physical activity (MET. hour/day) of the patients in GCE and placebo group did not have a significant difference at the initiation and the end of the trial.

According to Table 2, energy and nutrients intake of the participants were not significantly dissimilar between the two groups. As illustrated in Table 3, no discrepancy was observed in baseline quantities of dependent variables between the two groups. It can be inferred from the statistics that mean and standard deviation of IL-6 in GCE group decreased compared to the placebo group (-0.73 $\pm$ 2.65 in GCE group vs. 1.70 $\pm$ 10.51 (Pg/mL) in the placebo group); however, this attenuation was not statistically significant. Furthermore, hs-CRP values in the intervention group reduced by -0.28 $\pm$ 3.12 mg/L while its reduction in the control group was about -0.08 $\pm$ 4.15 mg/L. No significant difference was detected between the two groups in terms of hs-CRP level. In addition, the concentration of ICAM-1 in the intervention group attenuated by -0.05 $\pm$ 0.45 ng/mL while it increased by 0.02 $\pm$ 0.45 ng/mL in the placebo group. The difference of ICAM-1 changes was not significant between the GCE and placebo group. On the other hand, MDA values in the intervention and placebo group mildly increased and no significant discrepancy existed between the two groups in terms of MDA changes during the study. Furthermore, the waist circumference of the participants consuming GCE reduced significantly compared to the placebo group (-2.40 $\pm$ 2.54 vs. -0.66 $\pm$ 1.17 cm, P = 0.009). In addition, weight reduction of the GCE group was marginally significant compared with the placebo group (weight: -2.08 $\pm$ 2.11 vs. -0.92 $\pm$ 1.30 kg, P = 0.05).

5. Discussion

This clinical trial was carried out to explore the impacts of 800 mg/day of GCE supplementation for eight weeks on oxidative stress, vascular, and systemic inflammation in patients with metabolic syndrome. Alterations in serum levels of ICAM-1, MDA, hs-CRP, and IL-6 in GCE group were not significant in comparison with the placebo group in this trial. Waist circumference reduction in the GCE group was significantly greater than the placebo group. In addition, weight reduction in the intervention group was marginally significant compared to the placebo group.

Green coffee extract in our study suppressed IL-6 elevation compared to the placebo; however, changes of IL-6 did not differ significantly between the two groups. In the study by Song et al. (11), a significant decline in plasma IL-6 level was noted in mice fed 0.3% GCE (300 mg green coffee extract/kg diet) compared to HFD fed group after 11 weeks. This significant effect on IL-6 may be due to the fact that the amount of GCE used for mice in this study was equal to 1460 mg/60 kg GCE for humans, which is higher than the GCE dose used in our study. In addition, in the study of Hwang et al. (18), mice were injected three times with lipopolysaccharide (LPS) with or without 0.1 mg CGA (5 mg/kg) for three days, and CGA could decrease IL-6 mRNA levels dose-dependently by down- regulating nuclear factor κB (NF-κB). Wu et al. (15), exhibited that administration of 400 mg/kg CGA for 12 weeks significantly attenu-

Table 1. General Characteristics of Participants in Both Study Groups[a]

Variable	GCE Group, N = 21	Placebo Group, N = 22	P Value[b]
Gender			0.52
Female	17 (81)	16 (72.7)	
Male	4 (19)	6 (27.3)	
Smoking status			0.52
Yes	2 (9.5)	1 (4.5)	
No	19 (90.5)	21 (95.5)	
Oral hypoglycemic drugs			
Biguanides	19 (90.5)	15 (68.2)	0.07
Sulfonylureas	7 (33.3)	9 (40.9)	0.60
Alpha glucosidase inhibitors	4 (19)	3 (19.6)	0.63
Other	1 (4.8)	0 (0)	0.30
None	2 (9.5)	7 (31.8)	0.07
Lipid lowering drugs			
Statins	12 (57.1)	13 (59.1)	0.89
None	9 (42.9)	9 (49.9)	
Anti-hypertensive drugs			
Angiotensin II receptor antagonists	7 (33.3)	5 (22.7)	0.43
ACE inhibitors	2 (9.5)	3 (13.6)	0.67
Diuretics	1 (4.8)	2 (9.1)	0.57
Beta blockers	3 (14.3)	5 (22.7)	0.47
Other	1 (4.8)	1 (4.5)	0.97
None	10 (45.4)	9 (42.8)	0.65
Menopause status			
Yes	12 (57)	10 (45)	0.72
No	5 (23)	6 (27)	
Age (y)	52.76 ± 9.83	51.95 ± 8.67	0.77
Duration of diabetes (y)	3.83 ± 4.19	3.28 ± 4.29	0.67

Abbreviations: ACE inhibitors, angiotensin converting enzyme inhibitors; GCE, green coffee extract.
[a] Data are expressed as No. (%) (by Chi-square test) except for quantitative variables of age and duration of diabetes which are shown as Mean ± SD.
[b] P values are for comparison of the variables between the two groups (all by Chi-square test, except for age and duration of diabetes which were analyzed by independent *t*-test).

ated serum levels of IL-6 in ApoE$^{-/-}$ mice compared to the control group. Nevertheless, in one study, high dose infusion of CGA (7 mg/kg) for seven days resulted in increasing IL-6 and TNF-α level compared with the control group in rats, however, low dose of 0.3 mg/kg did not yield any notable effect on these biomarkers (25). Moreover, the the alterations in serum levels of hs-CRP in our study were not significantly different between the GCE and placebo group after the intervention. Up to our knowledge, our trial was the first study investigating the GCE supplementation effect on hs-CRP concentration.

In addition, our clinical trial demonstrated that GCE could inhibit elevation of ICAM-1 level as an indicator of vascular inflammation; however, no significant discrepancy was observed concerning ICAM-1 changes during the trial compared to placebo group. The previous studies have solely examined CGA impact rather than GCE on the level of adhesion molecules. In one study, administration of 25 and 50 μmol CGA was capable of suppressing mRNA expression of VCAM-1, ICAM-1, and endothelial cell selectin in a dose-dependent manner in IL-1beta-stimulated cells. Furthermore, these amounts of CGA could inhibit nuclear translocation of NF-κB subunits p50 and p65, which led to the reduction of cell adhesion molecules expression (20).

Our study exhibited no significant discrepancy between serum MDA changes in the intervention and placebo group at the end of the trial. This finding is inconsistent with some studies carried out previously. One study

Table 2. Dietary Intakes and Physical Activity of Participants in Both Study Groups[a,b,c]

Variable	GCE Group, N = 21	Placebo Group, N = 22	P[1]	P[2]	P[3]
Energy (Kcal/d)			0.5	0.63	0.92
Baseline	1601 ± 513	1692 ± 362			
4th week	1657 ± 443				
8th week	1515 ± 362	1595 ± 406			
1527 ± 424					
Carbohydrate (g/d)			0.42	0.51	0.96
Baseline	213.1 ± 61.6	230.1 ± 74.1			
4th week	218.6 ± 67.9	233 ± 77.3			
8th week	215.1 ± 70.5	216.2 ± 91			
Protein (g/d)			0.20	0.51	0.96
Baseline	52.4 ± 18.1	59.3 ± 16.6			
4th week	58.8 ± 26.8	63.2 ± 16.4			
8th week	56.4 ± 15.8	58 ± 16.4			
Fat (g/d)			0.78	0.12	0.8
Baseline	50.4 ± 20.8	48.9 ± 12.7			
4th week	54.6 ± 20.7	46.4 ± 12.1			
8th week	47.4 ± 18.9	46.4 ± 15.1			
Fiber (g/d)			0.31	0.15	0.16
Baseline	11.5 ± 5.5	13.1 ± 4.8			
4th week	12.2 ± 6.4	14.7 ± 4.8			
8th week	12.7 ± 4	13.3 ± 6.5			
Selenium (mg/d)			0.52	0.4	0.78
Baseline	0.05 ± 0.04	0.05 ± 0.03			
4th week	0.04 ± 0.03	0.05 ± 0.02			
8th week	0.05 ± 0.03	0.05 ± 0.03			
Vitamin C (mg/d)			0.71	0.2	0.47
Baseline	70.43 ± 38.26	70.23 ± 35.59			
4th week	74.67 ± 41.87	75.15 ± 46.93			
8th week	72.97 ± 39.52	77.70 ± 70.62			
Vitamin E (mg/d)			0.87	0.11	0.75
Baseline	2.02 ± 1.28	2.08 ± 1.19			
4th week	1.78 ± 1.13	2.36 ± 1.20			
8th week	3.15 ± 3.81	2.87 ± 1.43			
Physical activity (Met.hour/day)			0.12	-	0.58
Baseline	38.06 ± 4.59	40.25 ± 4.48			
8th week	38.21 ± 3.32	38.92 ± 4.97			

[a] P^1 P values are for comparison between the two groups at baseline (independent *t*-test).
[b] P^2 P values are for comparison between the two groups after 4 weeks (independent *t*-test).
[c] P^3 P values are for comparison between the two groups after 8 weeks (independent *t*-test).

compared green coffee extract effects with dark and light roasted coffee in diabetic rats. Green and light- roasted coffee administration resulted in attenuation of plasma MDA levels after four weeks (23). Hao et al. (14), conducted a study to examine the CGA effect on cadmium-induced oxidative stress in rats. Administration of 60 mg/kg CGA could diminish lipid peroxidation by reducing the MDA level in the brain, which had increased by cadmium exposure compared to rats exposed to cadmium alone after 30 days. A recent in vitro study investigated the effects of CGA on hydrogen peroxide-stimulated oxidative stress in osteoblast cells. The MC3T3-E1 cells were treated with different doses of CGA (0, 25, 50, 100 μM) for three hours and subsequently, they were exposed to hydrogen peroxide (H_2O_2). CGA treatment significantly suppressed oxidative stress induced by H_2O_2 by reversing the MDA levels

Table 3. Means and Standard Deviations of Dependent Variables Before and After the Study and Their Changes Throughout the Trial in Both Groups[a,b,c]

Variables	GCE Group, N = 21	Placebo Group, N = 22	P[1]
IL-6 (Pg/mL)			
Before	8.31 ± 2.85	9.03 ± 2.68	0.4
After	7.58 ± 1.74	10.74 ± 10.06	0.16
P[2]	0.21	0.45	
Changes	-0.73 ± 2.65	1.70 ± 10.51	0.3
hs-CRP (mg/L)			
Before	5.99 ± 4.96	5.87 ± 3.86	0.93
After	5.70 ± 4.94	5.79 ± 5.03	0.96
P[2]	0.86	0.92	
Changes	-0.28 ± 3.12	-0.08 ± 4.15	0.86
MDA (μmol/L)			
Before	6.15 ± 1.70	5.84 ± 1.87	0.57
After	6.60 ± 1.42	6.17 ± 2.090.5	0.44
P[2]	0.23		
Changes	0.44 ± 1.68	0.32 ± 2.28	0.84
ICAM-1 (ng/mL)			
Before	2.73 ± 0.78	2.70 ± 0.66	0.86
After	2.68 ± 0.61	2.72 ± 0.72	0.82
P[2]	0.56	0.78	
Changes	-0.05 ± 0.45	0.02 ± 0.45	0.54
Weight (Kg)			
Before	78.10 ± 11.01	80.11 ± 12.45	0.6
After	76.01 ± 10.52	79.18 ± 12.75	0.4
P[2]	0.000	0.009	
Changes	-2.08 ± 2.11	-0.92 ± 1.30	0.04
Waist circumference (cm)			
Before	106.4 ± 9.73	108.66 ± 10.43	0.4
After	104 ± 8.82	108 ± 10.12	0.2
P[2]	0.000	0.028	
Changes	-2.40 ± 2.54	-0.66 ± 1.17	0.009

Abbreviation: GCE, green coffee extract.
[a] Values are expressed as Mean ± SD.
[b] P[1]: obtained from Independent sample *t*-test.
[c] P[2]: obtained from paired *t*-test.

dose-dependently (30). One study indicated that high dose of CGA (7 mg/kg) in rats could significantly elevate MDA levels after seven days while the low dose (0.3 mg/kg) did not alter the MDA level compared to the control group (25).

In our study, GCE supplementation significantly led to the reduction of waist circumference. In addition, the difference between weight reduction in the GCE and placebo group was marginally significant. Some other researches have exhibited anti-obesity feature of GCE. A total of 600 mg of decaffeinated GCE supplemented for 40 days could lead to 1.36 kg weight loss in a pilot clinical study on 15 patients (31). In a crossover study, which compared 40 g of black and green coffee, a significant reduction in weight and BMI was noted for green coffee consumption. Waist circumference and abdominal fat of the participants also attenuated after consumption of both coffees. However, these effects could be ascribed to the caffeine content of the coffees while GCE capsules in our trial were decaffeinated (32). Furthermore, a clinical trial, which was carried out on 30 overweight subjects demonstrated a significant weight loss of about 5 kg after consumption of 11 g of a GCE enriched coffee (supplying 1000 mg GCE and 500 mg GCE per day) compared to the instant coffee group (33). The higher dose of GCE and longer duration of administration might have caused greater weight loss in this trial compared to our study. The study by Song et al., has pro-

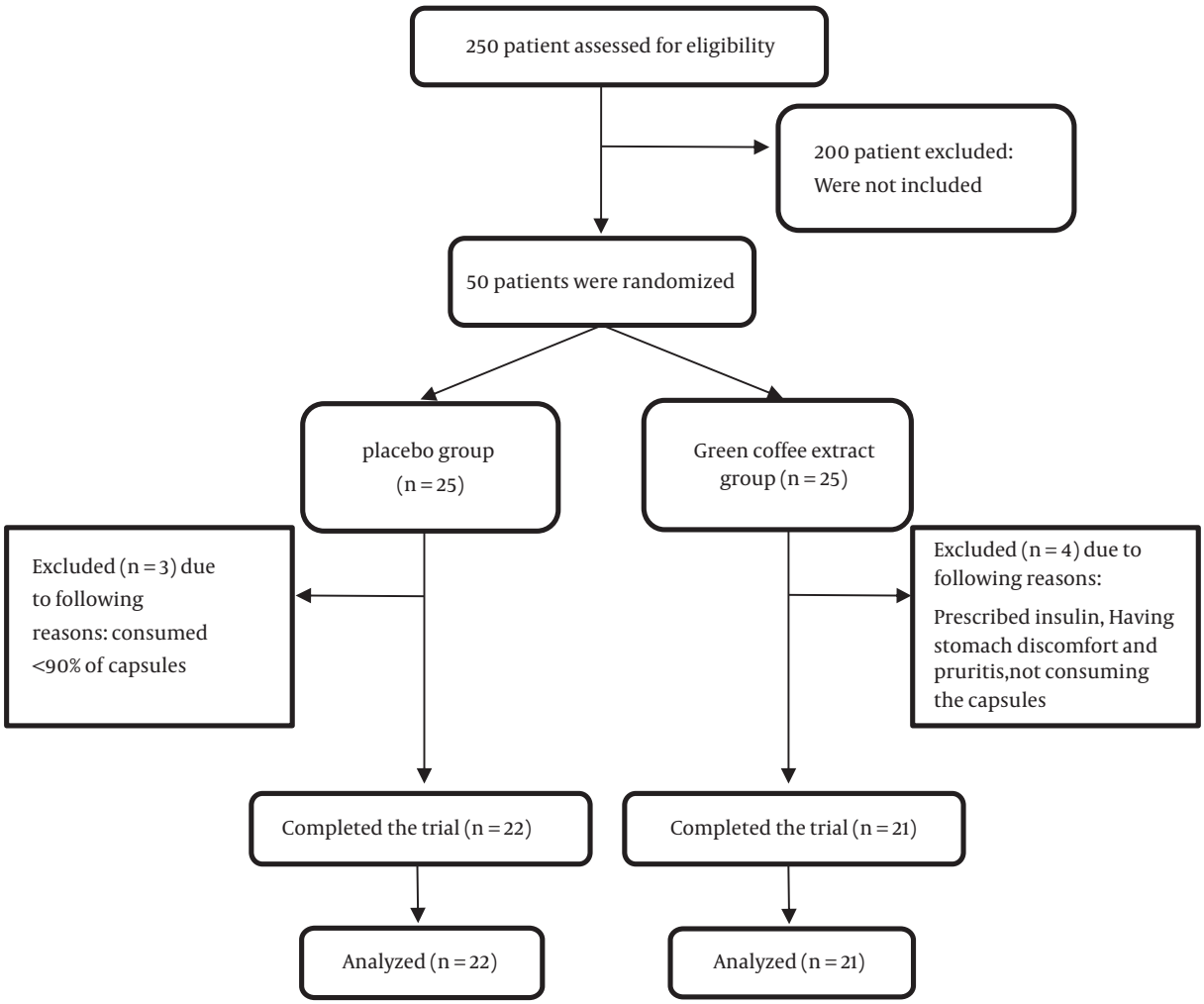

Figure 1. The patient flow diagram

posed that the anti- obesity property of GCE might be attributed to its effects on inhibiting adipogenesis, which is primarily regulated by transcription factors such as peroxisome proliferator-activated receptor γ^2 (11).

The limitations of our trial were the short follow- up period of the study. Moreover, we did not perform cell- based gene expression evaluation on our patients. Our study, for the first time, investigated the effect of oxidative stress and inflammation in patients with metabolic syndromes, more studies are needed to confirm our findings.

5.1. Conclusions

Finally, our findings, for the first time, showed that supplementation of GCE did not significantly alter serum levels of oxidative stress (MDA) and also inflammatory factors (ICAM-1, hs-CRP or IL-6). Therefore, in our study, GCE could not affect oxidative stress, systemic, and vascular inflammation in patients with metabolic syndrome. However, GCE could significantly reduce waist circumference. In addition, its effect on weight reduction was also marginally significant.

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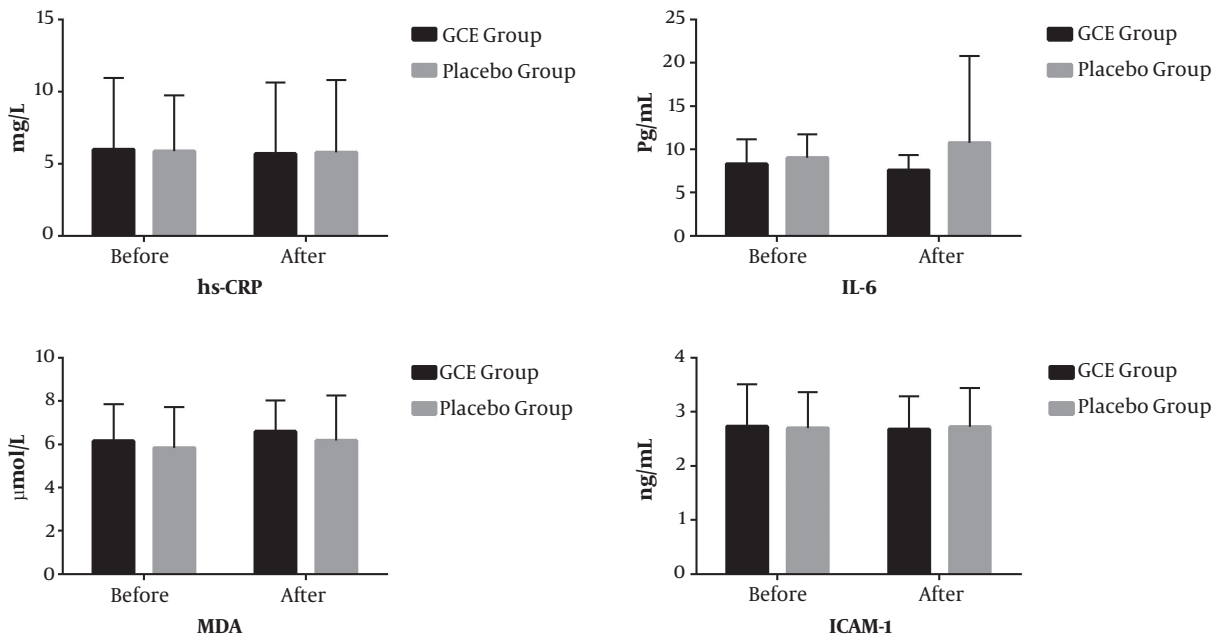

Figure 2. Mean ± SD of hs-CRP, IL-6, MDA and ICAM-1; Abbreviations: GCE, Green Coffee Extract; hs-CRP, High Sensitivity C-reactive Protein; IL-6, Interleukin-6; MDA, Malondialdehyde Acid; ICAM-1, Intracellular Adhesion Molecule-1; GCE, Green Coffee Extract.

Footnotes

Conflicts of Interests: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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