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Original article

Effects of green coffee extract supplementation on blood pressure and antioxidants status in patients with non-alcoholic fatty liver disease

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Summary. *Background and aim:* Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder. Hypertension and increased oxidative stress are important risk factors for NAFLD and its complications such as cardiovascular disease. This study aimed at assessing the effects of green coffee extract (GCE) on blood pressure (BP) and antioxidants status in patients with NAFLD. *Methods:* This double-blind, randomized, controlled clinical trial was conducted on 44 individuals with NAFLD aged 20-60 for male & 20-50 years for females and body mass index (BMI) ranged 25 - 34.99 kg/m^2. The intervention group (n=21) consumed GCE capsules (200 mg) two time per day for 8 weeks. The control group (n=23) received two placebo capsules daily for the same period. Anthropometric and BP measurements, dietary intakes, physical activity levels, ultrasonography and fasting blood samples were collected at the baseline and at the end of the trial. Data were analyzed by independent t test, paired t test and analysis of covariance. *Results:* GCE supplementation significantly reduced systolic BP (SBP) (mean difference = -9.62 and 95% confidence interval = -14.98 to -4.26), and BMI of subjects compared to control group at the end of study. Diastolic BP decreased significantly within GCE group and serum MDA increased within control group after intervention compared to their baseline measurements ($P < 0.05$). Changes in total anti-oxidant capacity were not significant in any of groups. *Conclusion:* GCE supplementation improved SBP and BMI in NAFLD patients. Further studies are warranted to evaluate more effects of GCE on NAFLD management.

Key words: non-alcoholic fatty liver disease, green coffee extract, blood pressure, antioxidants status

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder around the world encompassing a spectrum of conditions from simple steatosis and nonalcoholic steato-hepatitis to fibrosis and cirrhosis (1-6). It occurs when fat is deposited in the liver due to causes other than excessive alcohol use or viral and autoimmune disease (7-10). The prevalence in general population is estimated to be 10-35% and 5-30% in Asia (11-13). NAFLD is closely linked with components of metabolic syndrome including hypertension (HTN). Also, development of HTN is associated with higher incident cardiovascular disease (CVD) in NAFLD (4, 10, 14-16). Which explains increased mortality from CVDs among these patients (17).

The underlying mechanisms leading to NAFLD are not fully understood and it is considered to be a multifactorial disorder (18). However, oxidative stress accounts as one of the most critical factors in NAFLD pathogenesis and the progress of CVD in this disorder (17, 19). Elevated levels of reactive oxygen species

(ROS), lipid oxidation products and low levels of antioxidant enzymes have been reported in subjects with NAFLD (20).

Despite the prevalence of NAFLD, no effective treatment has been recognized for this disorder. The current strategies for its management includes dietary modification, physical activity and lifestyle changes (21, 22). Recently, the effect of nutraceuticals on NAFLD has received much attention (23-25). One promising nutraceutical is green (or raw) coffee (26, 27). It is one of the popular consumed beverages in recent years (26).

Green coffee encompasses essential nutrients including monosaccharides, amino acids and fatty acids as well as some vitamins and minerals. It is also a good source of antioxidant integrates such as chlorogenic acids (CGAs), hydroxycinnamic acids and caffeic acid (26). Green coffee extract (GCE) is a rich source of CGA, neochlorogenic acid, feruloylquinic acid, cafestol, kahweol, tocopherols and trigonelline (28-31). Beneficial effects of GCE on body weight (32, 33), vaso-reactivity (34), HTN (30, 35) and glucose metabolism (36) have been reported in some animal and human studies. Also, anti-oxidant properties have been evidenced in some studies (27, 37-39). However, limited data regarding possible effects of GCE in patients with NAFLD are available. Based on the assumption that GCE may affect above mentioned risk factors in NAFLD patients, we aimed to investigate the effects of GCE on blood pressure (BP) and antioxidant status (serum total anti-oxidant capacity (TAC) and malondialdehyde (MDA)) in these patient.

Materials and Methods

Participants

This double-blind, randomized controlled clinical trial was conducted among 44 adult patients with NAFLD recruited from 22 Bahman polyclinic in Neyshabur, Iran. All subjects underwent ultrasonography for diagnosis of NAFLD and its grades by a single sonographist. Echogenicity grading was carried out according to macrovesicular steatosis and divided into 4 categories: no steatosis, grade 1: up to 33% steatosis, grade 2: 33%–66% steatosis, and grade 3: > 66% steatosis (40). The inclusion criteria were aged of 20-60 in men and 20-50 in women and body mass index (BMI) of 25-34/9 kg/m^2. The participants were excluded if they suffered from diabetes, pulmonary disease, renal disease, liver transplantation and chronic or acute liver disease (Hepatitis A, B, ...), inherited disorders affecting liver condition, biliary impairments, autoimmune disease, wilson disease, hemochromatosis, cancers, inflammatory bowel diseases, thyroid disorders, history or current diagnosis of CVD, gastrointestinal disease. Also the presence of alcohol abuse, smoking, pregnancy, lactation and menopause, management for weight control and anemia were considered as exclusion criteria. Athletes or very active subjects and those with regular exercise regimens, individuals taking medications such as hepatotoxic agents, anticoagulants or dietary supplements, estrogen and oral contraceptive drugs, herbal preparations and those with allergy to coffee or its components were also excluded. The study protocol was completely described to the patients and their informed written consent was obtained.

Ethics statements

The trial was confirmed by the ethics committee of Tabriz University of Medical Sciences (TBZMED.REC.1394.1031) and was registered in the Iranian Registry of Clinical Trials (IRCT number: 201602113664N17). The study was consistent with the guidelines of the Declaration of Helsinki.

Sample size

Considering $\alpha = 0.05$ and a power of 80%, the sample size was calculated to be 22 subjects per group based on the information for alanine transaminase (ALT) obtained from the study by Ebrahimi-Mameghani et al. (7). This number was enhanced to 24 in each group to accommodate for the anticipated dropout rate.

Randomization

The Participants were randomly assigned to either GCE or control groups using a block randomization procedure (of size 4) with matched subjects in each block based on sex, age (< 40 and >40 years) and BMI (25-29.99 and 30-34.99). Randomization was carried out using random allocation system (RAS) 9.3 (RAS Institute Inc., 2012, Cary, NC, USA) (41).

Study design

Patients in GCE group (n= 24) received two 200 mg GCE capsules/day (one capsule after breakfast and lunch), for eight weeks while those in the control group (n= 24) received two capsules of placebo /day for the same period of time. The GCE capsules were purchased from Bonyan Salamat kasra co (Tehran, Iran), made from green coffee beans by hydro-alcoholic extraction method. They contained 50 percent CGA as the main ingredient with low levels of caffeine (<2%). Placebo capsules were made of starch and were similar to the GCE capsules in dosage, size and color. All of the supplement packs were identical looking. GCE and placebo capsules were distributed every two weeks. Weekly follow ups were carried out by telephone and the number of returned supplement packs were checked every two weeks. Healthy lifestyle recommendations were provided to the patients at the beginning of the study. Patients and those involved in recruitment process, administering interventions, and assessing outcomes were blind to group assignments.

Nutritional assessment

Dietary data was collected using a three-day dietary record method (two weekdays and one weekend day) and averages of three-day energy and macronutrient intakes was analyzed using Nutritionist IV Software (First Databank Inc., San Bruno, CA).

Physical activity

Physical activity was assessed by the international physical activity questionnaire (IPAQ) (42).

Anthropometric measurements

Body Weight and height were measured using Seca scale (Hamburg, Germany) with accuracy 0.5 kg and 0.5 cm, respectively. Individuals were without shoes and wearing light clothing. BMI was calculated as weight (kg)/ height^2 (m).

BP measurements

Systolic BP (SBP) and diastolic BP (DBP) were evaluated in the right upper arm by a mercury sphygmomanometer while subjects were sitting and had rested for at least five minutes before measurements. SBP and DBP were assayed as the first detectable sound (phase I), and the disappearance (phase V) of Korotkoff sounds, respectively. Two readings were recorded at an interval of one to two minutes. The mean of two readings was calculated for analysis.

Blood sampling and laboratory assays

Venous blood samples (5 ml) were taken after a 12 h overnight fasting in the morning. The serum samples were separated from whole blood and stored in −70°C until assay time. Blood samples were analyzed at the Drug Applied Research Center (Tabriz University of Medical Sciences, Tabriz, Iran). The serum MDA level was measured based on reaction with thiobarbituric acid as a thiobarbituric acid reactive substance to produce a pink colored complex. Next, its fluorescence intensity was measured at 547 nm with excitation at 525 nm using a spectrofluorimeter (model SFM 25 A; Kontr (43). Serum TAC was assayed by using the colorimetric method with commercial kits (RANDOX kits; UK) (44).

Measurement of ALT and aspartate transaminase (AST) were conducted by standard enzymatic methods using a Pars Azmoon kit (Karaj, Iran).

All ultrasonography, 3-day food record, physical activity, anthropometric, BP and biochemical measurements were assessed again at the end of the trial period for both groups.

Statistical analyses

Statistical analysis was performed by SPSS software (version 18; SPSS Inc., Chicago, IL) and the results were expressed as mean ± standard deviation (SD) or number (percent). The normality of the distribution of variables was determined with Kolmogorov–Smirnov test. The baseline measurements and dietary intakes of subjects were compared using independent samples t-test and chi-squared tests. The changes in dietary intakes, BP, anthropometric measurements and serum parameters over the study period in each group were assessed using paired t-test.

Analysis of covariance (ANCOVA) was used to identify any differences between the 2 groups after trial, adjusting for baseline measurements and confounders (BMI and energy changes during study). Percent of changes in variables after the intervention was calculated by the formula ([after values - before values] divided by before values) × 100. $P < 0.05$ was considered to be statistically significant.

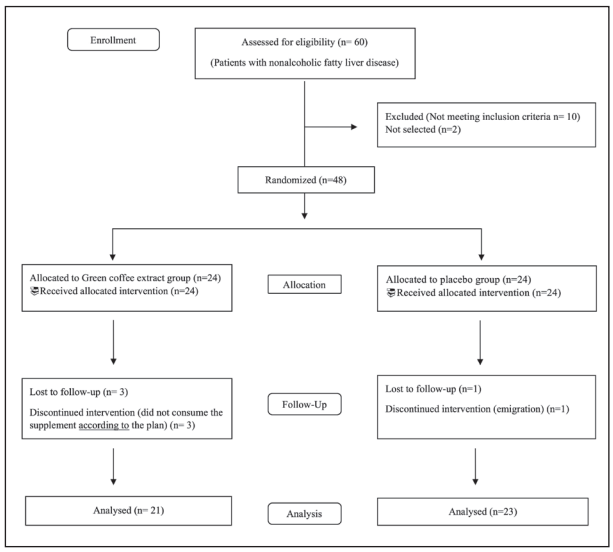

Figure 1. Flow diagram of the study

Results

Patient inclusion, study completion, safety and compliance

A total of 48 NAFLD patients participated in the present clinical trial. Four subjects dropped out of the study for not consuming the supplements according to the plan and immigration. Thus, 21 individuals in the GCE group and 23 individuals in the control group completed the study (Fig. 1). Compliance was good with more than 94 % of the GCE capsules being consumed during the study period. No adverse effects or symptoms caused by GCE were recorded.

General characteristics

Table 1 shows the general, anthropometric and dietary intake of subjects. No significant differences were observed between the two groups in terms of age, sex,

Table 1. General and clinical characteristic of the patients with NAFLD

Variable	Green coffee extract group (n=21)	Control group (n=23)	P value
Age (year)			
Mean (SD) *	41.14 ± 7.87	41.13 ± 8.47	0.996 [a]
Range	24-53	27-60	
Gender			0.989 [b]
Male	11 (52.4%)	12 (52.2%)	
Female	10 (47.6%)	11 (47.8%)	
Weight (kg)			
Baseline	85.95 ± 11.76	83.97 ± 10.59	1.97 (-4.82 to 8.77), 0.561 [a]
After intervention	84.30 ± 12.17	83.60 ± 11.06	-1.73 (-2.44 to -1.01), <0.001 [c]
MD (95 % CI),P value	-1.64 (-2.11 to -1.18),<0.001 [d]	-0.36 (-0.91 to 0.17),0.175 [d]	
BMI (kg/m^2)			
Baseline	30.14 ± 2.6	30.51 ± 2.81	-0.37 (-2.02 to 1.28), 0.653 [a]
After intervention	29.54 ± 2.59	30.37 ± 2.96	-0.57 (-0.84 to -0.29), <0.001 [c]
MD (95% CI),P value	-0.60 (-0.77 to -0.42), <0.001 [d]	-0.13 (-0.32 to 0.04),0.14 [d]	
Grade of fatty liver			
Baseline			0.310 [b]
Normal	(n= 0) 0%	(n= 0) 0%	
Grade1	(n= 12) 57.1%	(n= 15) 65.2%	
Grade 2	(n= 8) 38.1%	(n= 7) 30.4%	
Grade 3	(n= 1) (4.8%)	(n= 1) 4.3%	
Baseline physical activity			0.069 [b]
Low/ Moderate/ Vigorous	5 (24%)/ 8 (38%)/ 8 (38%)	10(43.5%)/2(8.5%)/11(49%)	

*n (%); NAFLD, nonalcoholic fatty liver disease nonalcoholic fatty liver disease; * The results are described as mean ± standard deviation (SD) [a] Data are tested by independent sample t test; [b] Data are tested by chi-square test; [d] Data are tested by paired sample t test; [c] Data are tested by covariance adjusted for energy intake and baseline values*

weight, BMI, grade of fatty liver, physical activity and daily energy and macronutrients intakes at the baseline.

Significant decreases in the mean of weight and BMI were observed in the GCE group after the intervention compared to the baseline values (P < 0.001). Results of ANCOVA showed significant differences between the two groups in weight and BMI at the end of the study adjusted for energy intake and baseline values (P> 0.001).

Dietary intakes

Daily energy and macronutrients intakes of participants at the beginning and end of the study are reported in Table 2. No significant differences were seen in energy or macronutrient intakes between the 2 groups at baseline. Energy and macronutrients intakes of studied subjects did not change significantly within or between groups after intervention in both groups.

BP measurement and biochemical parameters

Table 3 presents SBP, DBP and biochemical variables of participants at the baseline and after 8 week intervention. No significant differences were seen between the two groups in SBP, DBP, serum TAC and liver enzymes at the beginning of the study. Serum levels of MDA were significantly different between the two groups at the baseline (P=0.004).

The mean of SBP and DBP in the GCE group significantly decreased at the end of the study compared to the baseline values (by 9.2%, P < 0.001; by 7.8%, P < 0.001, respectively). Serum levels of MDA elevated significantly within placebo group at the end of the study (by 31.6%, P= 0.001).

Results of ANCOVA showed significant differences between the two studied groups in SBP at the end of study, adjusted for BMI, energy intake and baseline values (P = 0.001). Changes in other variables were not significant between two groups (P>0.05).

Discussion

NAFLD is a major challenge to the healthcare systems around the world. Most individuals with this disorder do not develop severe liver disease but have an enhanced risk of CVD. Thus, the treatment of

Table 2. Dietary intakes of the patients with NAFLD at baseline and after 8 weeks of intervention

Variable	Green coffee extract group (n=21)	Control group (n=23)	MD (95 % CI), P value
Energy (kcal/day)			
Baseline	2325.95 ± 326.88	2315.00 ± 469.37	10.95 (-237.43 to 259.33), 0.930 [a]
After intervention	2330.95 ± 313.2	2301.34 ± 468.8	18.87 (-10.45 to 48.20), 0.201[c]
MD (95% CI), P value	-5.00 (-34.87 to 24.87), 0.963 [b]	13.65 (-10.04 to 37.34), 0.388 [b]	
Carbohydrate (g/day)			
Baseline	253.19 ± 54.19	254.04 ± 75.35	-0.85 (-41.13 to 39.42), 0.966 [a]
After intervention	260.04 ± 47.4	250.91 ± 73.6	9.86 (-7.10 to 26.82), 0.247 [c]
MD (95% CI), P value	-6.85 (-28.18 to 14.46), 0.797[b]	3.13 (-6.94 to 13.21), 0.816[b]	
Protein (g/day)			
Baseline	71.32 ± 22.45	68.73 ±15.77	2.58 (-9.13 to 14.31), 0.658 [a]
After intervention	67.00 ± 19.2	67.23 ± 17.3	-2.17 (-9.17 to 4.82), 0.534 [c]
MD (95% CI), P value	4.32 (-3.98 to 12.64), 0.469[b]	1.50 (-3.76 to 6.76), 0.850[b]	
Total fat (g/day)			
Baseline	116.09 ± 20.15	116.32 ± 23.62	-0.23 (-13.65 to 13.19), 0.972 [a]
After intervention	118.28 ± 17.8	115.10 ± 20.8	3.33 (-3.98 to 10.66), 0.363[c]
MD (95% CI), P value	-2.19 (-11.57 to 7.19), 0.909[b]	1.21 (-4.29 to 6.72), 0.923[b]	

NAFLD, non-alcoholic fatty liver disease; MD, mean difference; The results are described as mean ± standard deviation (SD); [a] MD (95 % CI); P value is reported based on the analysis of independent sample t test; [b] MD (95 % CI); P value is reported based on the analysis of paired sample t test; [c] MD (95 % CI); P value is reported based on the analysis of covariance

Table 3. SBP, DBP and serum biochemical parameters of the patients with NAFLD at baseline and after 8 weeks of intervention

Variable	Green coffee extract group (n=21)	Control group (n=23)	MD (95 % CI), P value
SBP			
Baseline	120.90± 8.96	120.08±16.50	0.81(-7.19 to 8.83), 0.838 [a]
After intervention	109.76±12.06	118.69±15.69	-9.62(-14.98 to -4.26),0.001[c]
MD (95% CI), P value	-11.14(-14.83to -7.44),<0.001[b]	-1.39(-4.07to 1.28),0.293[b]	
DBP			
Baseline	82.61±7.06	82.13±12.25	0.48(-5.67 to6.65),0.874[a]
After intervention	76.14±8.13	79.17±11.14	-3.76(-8.36 to 0.82),0.105[c]
MD (95% CI), P value	-6.47(-9.11 to-3.83),<0.001[b]	-2.95(-6.10 to0.19),0.064[b]	
Serum TAC (nmol/ ml)			
Baseline	1.64±0.64	1.67±0.41	-0.03 (-0.36 to 0.29), 0.837 [a]
After intervention	1.60±0.63	1.66±0.54	-0.08 (-0.31 to 0.14),0.445 [c]
MD (95% CI), P value	-0.04 (-0.18 to 0.10), 0.566 [b]	-0.014 (-0.14 to 0.11), 0.817 [b]	
Serum MDA (nmol/ ml)			
Baseline	1.80±0.58	1.39±0.23	0.40 (1.39 to 0.67), 0.004 [a]
After intervention	2.00 ± 0.58	1.83 ± 0.56	-0.023 (-0.44 to 0.39), 0.913 [c]
MD (95 % CI), P value	0.20 (-0.08 to 0.49), 0.151 [b]	0.43 (0.21 to 0.66), 0.001 [b]	
ALT (IU/L)			
Baseline	43.85 ± 25.82	36.56 ± 19.19	7.29 (-6.47 to 21.05), 0.291 [a]
After intervention	44.52 ± 30.08	37.04 ± 19.99	8.65 (-6.93 to 24.2), 0.268 [c]
MD (95% CI), P value	0.66 (-10.99 to 12.32), 0.906 [b]	0.47 (-3.71 to 4.67), 0.815 [b]	
AST (IU/L)			
Baseline	35.71 ± 22.63	30.00 ± 9.39	5.71 (-4.66 to 16.09), 0.27 [a]
After intervention	32.66 ± 16.74	32.13 ± 11.14	1.53 (-8.42 to 11.50), 0.757 [c]
MD (95% CI), P value	-3.04 (-11.94 to 5.84), 0.483 [b]	2.13 (-1.76 to 6.02), 0.269 [b]	

NAFLD, non-alcoholic fatty liver disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; MD, mean difference; TAC, total antioxidant capacity; MDA, malondialdehyde; ALT, Alanine transaminase; AST, and aspartate transaminase; The results are described as mean ± standard deviation (SD); [a] MD (95% CI); P value is reported based on the analysis of independent sample t test; [b] MD (95% CI); P value is reported based on the analysis of paired sample t test; [c] MD (95% CI); P value is reported based on the analysis of covariance adjusted for BMI, energy intake and baseline values

NAFLD should be based on a global approach (45). It is also pertinent to recognize nutraceuticals that may help to counteract NAFLD (23, 24).

Based on the results, dietary intakes and physical activity level of studied subjects did not alter significantly in any of groups throughout the study period. As a result, these variables would not be considered as confounding factors in interpreting of the obtained outcomes.

Results regarding weight and BMI in the present study were in accordance with some previous animal and human works (29, 32, 46-50). Suppressed fat and glucose absorption and downregulated mRNA levels of adipogenic transcription factors have been suggested potential mechanisms involved in diminished adiposity by GCE (29, 32, 49).

The results of the present study also showed that GCE consumption abated SBP and DBP within the study groups and reduced SBP in comparison with control group. These findings are consistent with those of some previous works on green coffee and its components (30, 35, 51-54). Suzuki et al. reported that in spontaneous hypertensive rats (SHR), a single or long-term oral administration of GCE lowered BP in a dose-dependent manner. It was suggested that the hypotensive effect of ferulic acid in GCE might be mediated through the muscarinic acetylcholine recep-

tors (54). In another study Suzuki et al. demonstrated that CGA and caffeoylquinic attenuated HTN in SHR. Inhibition of ROS production in blood vessels and regulation of nitric oxide synthases that generate nitric oxide (NO) were suggested as contributing mechanisms. Caffeoylquinic may improve BP through direct scavenging of ROS and reducing superoxide anion production via the inhibition of NADPH oxidase activity. Such conditions regulate vascular tone or proliferation in SHR (53).

Studied carried out on healthy individuals (52) and mildly hypertensive subjects (35, 51) have indicated reduced SBP and DBP by consumption of green coffee, GCE or CGA (35, 51, 52). 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme existing in the adipose tissue and the liver involves in the conversion of hormonally inactive cortisone into active cortisol. Cortisol may decrease the production and bioavailability of vasodilators such as NO. It was shown that CGA in green coffee inhibits 11β-HSD1 activity (52).

CGAs might interact with the renin-angiotensin aldosterone system through inhibiting angiotensin-converting enzyme activity (55). Higher intake of Ca, K and Mg in the green coffee may also contribute to prevention of HTN (56).

A 5 mmHg decrease in SBP leads to lower stroke and CVD mortality by 14% and 9%, respectively (4). Each 2 mmHg decline in DBP could be associated with 14% fewer strokes and 8% less cases of CVD (35).

In the present study, serum MDA and TAC levels were not affected by GCE consumption. Therefore, favorable effects on SBP in the present study might not be mediated through antioxidant pathways. Li et al. also reported that, GCE did not have protective effects against increased oxidative stress caused by a high fat diet in a mouse model of metabolic syndrome (57). Suzuki et al. explained that caffeoylquinic in CGA did not change TAC in Wistar-Kyoto rats and SHR after 8 weeks (53). On the other hand, some studies have indicated that GCBE or CGA possess antioxidant activity in vitro and increase the antioxidant capacity of plasma in vivo (27, 37-39).

In the study by Baeza et al. GCBE and its main polyphenols protected human HepG2 cells against oxidative stress induced by tertbutylhydroperoxide. It was explained that polyphenols act as chelating agents, free radicals scavengers or modulators of endogenous antioxidants (37). Jung et al. investigated the protective effect of caffeic acid on oxidative damage induced by diabetes in C57BL/KsJ-db/db mice. Caffeic acid administration enhanced antioxidant enzyme activities and their mRNA levels in erythrocyte and liver of the animals (58).

In our study, serum MDA increased in control group at the end of study. However, no significant changes were detected regarding this variable within GCE group. It seems that, to some extent, GCE supplementation has protective effects against lipid peroxidation. Serum concentrations of liver enzymes and grade of NAFLD also did not alter after intervention. On the other hand, studies on mice have demonstrated that CGA shows favorable effects through different pathways such as lowering ALT and AST and anti-inflammatory properties (47, 59-61). Further studies are required to evaluate heptoprotective effects of GCE in humans.

Our study had some limitations such as short study duration and use of the ultrasonography technique instead of biopsy as the diagnostic criterion for NAFLD. Efficacy of GCE in controlling NAFLD warrants that more studies with different dose and duration are carried out.

Conclusion

The results of this study indicated that GCE supplementation had hopeful effects on SBP and BMI in patients with NAFLD. Further studies are suggested to examine its effects on controlling NAFLD risk factors.

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