



The Effect of Green Tea Extract Supplementation on Liver Enzymes in Patients with Nonalcoholic Fatty Liver Disease

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ABSTRACT

Background: Green tea is one of the most popular beverages in the world. It is believed to have beneficial effects in the prevention and treatment of many diseases, one of which is nonalcoholic fatty liver disease (NAFLD). The present study investigated the effects of consumption of green tea in NAFLD patients.

Methods: This study was a double-blind, placebo-controlled, randomized clinical trial. Ultrasonography was used to diagnose fatty liver in patients with alanine aminotransferase (ALT) >31 mg/dl and 41 mg/dl and aspartate aminotransferase (AST) >31 mg/dl and 47 g/dl in women and men, respectively and without other hepatic diseases. A total of 80 participants (20–50 years) with NAFLD were randomly allocated into two groups to receive either green tea extract (GTE) supplement (500 mg GTE tablet per day) or placebo for 90 days. At baseline and at the end of the intervention weight, serum ALT, AST, and alkaline phosphatase (ALP) were measured in fasting state, and dietary data were collected at baseline and end of the study.

Results: Green tea group showed significant reductions in ALT and AST levels after 12 weeks period ($P < 0.001$). The placebo group showed a reduction in ALT and AST levels at the end of the study, but it was no significant. ALP levels showed significant reductions in both groups after 12 weeks period ($P < 0.001$).

Conclusions: According to the findings of this study, GTE supplementation decrease liver enzymes in patients with NAFLD. It can be claimed that GTE prescribed can be considered as a treatment to improve serum levels of liver enzymes in NAFLD patients.

Keywords: Alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, green tea extract, nonalcoholic fatty liver disease

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a constellation of progressive liver disorders that are closely related to obesity, diabetes, and insulin resistance. NAFLD prevalence has increased with the change in eating habits, thus identifying an effective treatment for NAFLD is a significant public health objective.

Lifestyle-related factors such as poor diet, obesity, excessive alcohol intake, diabetes, and hyperlipidemia have all been proposed to contribute to NAFLD. In addition to the development of a fatty liver, NAFLD patients may also exhibit inflammation, necrosis and fibrosis of the liver, which are known as nonalcoholic steatohepatitis (NASH).^[1] This disease may progress to cirrhosis of the liver and hepatocellular carcinoma. Lifestyle interventions such as improvement of eating habits or physical activity are commonly recommended for NAFLD and NASH, but no effective medical therapy for these diseases has been established, although many medications for the treatment of NAFLD is undergoing clinical trials in the Western countries.

Green tea (*Camellia sinensis*) is one of the most popular beverages in the World. It contains high levels of flavonoids, which have antioxidant properties. Catechin, one of the main flavonoids in green tea has recently attracted attention for its anti-tumor^[2] and anti-arteriosclerotic^[3] effects. Catechins account for ~20% of the flavonoids in green tea leaves.^[4] The major tea catechins include epicatechin, epicatechin gallate, epigallocatechin (EGC), and EGC gallate (EGCG).^[5] They have been found to decrease oxidative stress^[6] and to exert anti-virus,^[7] anti-allergenic,^[8] anti-hypertensive,^[9] and anti-hyperglycemic effects.^[10,11] In addition, results of animal experiments have indicated that catechins affect the lipid metabolism by decreasing triglyceride and total cholesterol levels^[12] and enhancing energy utilization.^[13] Experimental evidence supports a role of green tea extract (GTE) or its catechins in protecting against NAFLD by regulating energy homeostasis and decreasing oxidative stress and inflammatory responses.^[14,15] However, the effect of the GTE on humans and its detailed mechanism have yet to be clarified. To the best of our knowledge, we conducted this randomized clinical trial to examine the effect of GTE on NAFLD in humans.

METHODS

This double-blind, randomized controlled clinical trial was carried out on 80 obese adult patients with NAFLD recruited from Metabolic Liver Disease Research Center in Isfahan University of Medical Sciences from September 2013 to February 2014. All subjects were randomly assigned to one of the two above-mentioned groups. A random number between 0.0 and 0.99 was generated by the computer for each subject. Subjects with a random number between 0.0 and 0.49 were assigned to the group with GTE, while those with a random number between 0.50 and 0.99 were assigned to the placebo group with cellulose. The same opaque capsules containing either dried powdered GTE or

placebo (cellulose) were administered to the subjects by a research assistant blinded to the contents in the capsules. All subjects were treated in the same fashion. The study protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences and was registered in the Iranian Registry of Clinical Trials website (IRCT2013092611763N12).

The sample size was computed 35 per group by considering $\alpha = 0.05$ and a power of 90%. This number was increased to 40 per group to accommodate the anticipated dropout rate. All participants underwent ultrasonography for determining fatty liver by a single sonographer. Echogenicity grading was performed using SonoAce X4 Medison (South Korea, Seoul). NAFLD is defined by elevated liver enzymes (alanine aminotransferase [ALT] >31 mg/dl and 41 mg/dl and aspartate aminotransferase [AST] >31 mg/dl and 47 g/dl in women and men, respectively) and echogenicity grading of the liver was based on Saverymattu *et al.*,^[16] that is, "mild steatosis" as a slight increase in liver echogenicity and exaggeration of liver and kidney echo discrepancy, and relative preservation of echoes from the walls of the portal vein, "moderate steatosis" as a loss of echoes from the walls of the portal veins, particularly from the peripheral branches, resulting in a featureless appearance of the liver as well as a greater posterior beam attenuation and a greater discrepancy between hepatic and renal echoes, and "severe steatosis" as a greater reduction in beam penetration, loss of echoes from most of the portal vein wall. The main branches and a large discrepancy between hepatic and renal echoes.

Of totally 153 recruited patients, only 80 confirmed NAFLD patients met the inclusion criteria Inc. aged 20–50 years of gender and body mass index (BMI) equal or over 30 kg/m². Patients with liver diseases such as Wilson's disease, autoimmune liver disease, hemochromatosis, virus infection, and alcoholic fatty liver as well as those with hepatotoxic, lipid lowering, metformin consumption and antihypertensive medication, contraceptive, and estrogen were excluded. The aim of the study was carefully explained to the patients, and their written informed consent was obtained. Personal characteristics Inc. demographic and disease history were obtained. Height and weight were measured by using standard protocols with participants in light clothes and without shoes. BMI was calculated as weight (kg) divided by height squared (m²), and body fat percentage was estimated with Omron body fat monitor (HBF-306) at baseline and at the end of the study. Dietary data were collected using a 3-day dietary record and averages of 3-day energy and macro-nutrients intakes were analyzed using Nutritionist

Software (Version 4.1, First Databank Division, The Hearst Corporation, San Bruno, CA). A self-reported questionnaire was used to estimate physical activity level.^[17] The subjects were randomly allocated into two groups “Green tea extract” and “placebo” groups.

Our GTE samples were prepared by Giah Essence Phytopharm Corporation (Gorgan, Iran). GTE samples were extracted from dried leaves of green tea according to the present standard procedures with a certificate of analysis given. The placebo given to the control group comprised pure microcrystalline cellulose. The placebo tablets supplied by School of Pharmacy, Isfahan University of Medical Sciences, Iran were similar to green tea tablets in color and size. The subjects were asked to take one capsule containing 500 mg of either GTE or cellulose each day for 12 weeks. The above capsule was taken 30 min after lunch. Table 1 shows the components of caffeine and polyphenols in the capsules.

All participants were asked to maintain their usual lifestyle. Blood samples were taken after 12–14 h of overnight fasting at baseline and after the intervention period. Serum samples were transferred into microtubes and were stored at -70°C until analysis. ALT and AST were determined by the method developed by International Federation of Clinical Chemistry.^[18,19] Alkaline phosphatase (ALP) was determined by Deutschen Gesellschaft für Klinische Chemie.^[20] All statistical procedures were performed using SPSS Software (Version 16, Spss Inc. Chicago, IL). Normality of continuous variable was tested by Kolmogorov–Smirnov test. Data are expressed as mean \pm standard deviation (SD) for normally distributed variables. Differences in the mean of the continuous variables between the two groups were tested using analysis of covariance for adjusting for baseline measurements and covariates. Changes in biochemical parameters over the study period were estimated by after intervention minus

baseline amount was done using paired *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Of totally 80 patients, 71 patients completed the study ($n = 35$ and 36 for green tea and placebo groups, respectively) [Figure 1]. General characteristics of the study subjects are shown in Table 2. There were no significant differences at baseline between the two groups in terms of age, gender, marital status, education level, physical activity, and the severity of fatty liver. The mean daily macronutrient and dietary fiber intakes were shown in Table 3. Dietary data assessment over the study showed no significant changes in total energy intake, fat, protein, and dietary fiber over the study in both groups. There was a significant reduction in carbohydrate intake in green tea group ($P = 0.02$). There were no significant differences in the mean nutrient intakes between the two groups at baseline and also after 12 weeks. The mean \pm SD of biochemical factors before and after intervention are shown in Table 4. At the beginning of the study, the groups were similar based upon weight (87.22 ± 11.75 kg and 90.51 ± 14.88 kg in intervention and placebo group, respectively) and liver enzymes. Weight decreased significantly in both groups ($P < 0.001$). The mean weight change in the green tea group was significantly greater than the placebo group ($P = 0.001$). Green tea group showed significant reductions in ALT and AST levels after 12 weeks period ($P < 0.001$). The placebo group showed

Table 1: Components of caffeine and polyphenols (500 mg each capsule)

Components	Percentage of weight	
	Green tea extract	Placebo
Caffeine	2.275	0
GC	5.132	0
EGC	3.076	0
Catechins	0.690	0
EC	5.861	0
EGCG	31.429	0
GCG	2.290	0
ECG	2.647	0
Cellulose	46.60	100

GC=Gallic acid, EGC=Epigallocatechin, EC=Epicatechin, EGCG=Epigallocatechin gallate, GCG=Gallic acid gallate, ECG=Epicatechin gallate

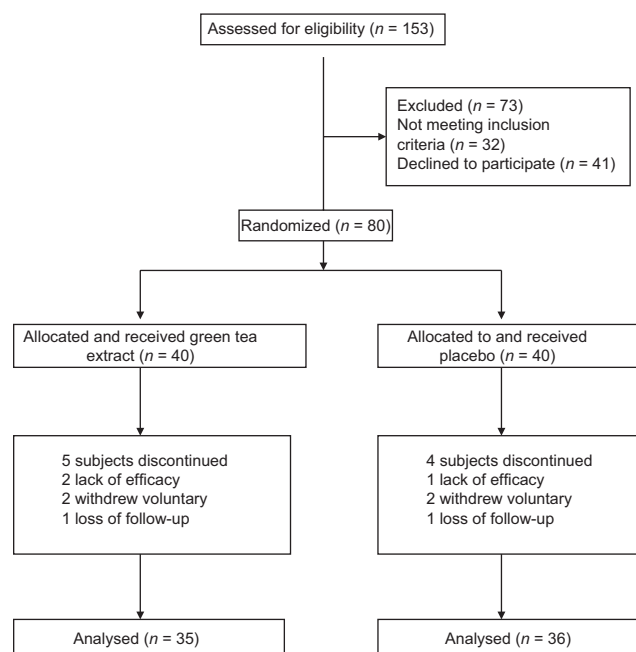


Figure 1: Study flow diagram

Table 2: Baseline characteristics of the NAFLD patients in each group

Characteristic	Green tea extract (n=35) n (%)	Placebo (n=36) n (%)	Significant (χ^2)
Male	16 (52.8)	16 (58.8)	0.789
Married	28 (94.2)	24 (89.6)	0.661
Less than high school	10 (32)	15 (54.9)	
Completed high school	15 (49.3)	9 (31.77)	0.853
University certificate	7 (21.69)	50 (16.39)	
Physical activity*			
Inactive	12 (38.9)	12 (43.4)	0.755
Light activity	9 (25.2)	9 (31.9)	
Moderate activity	12 (38.9)	8 (27.9)	
Severity of fatty liver**			
Mild	15 (49.4)	18 (66.5)	0.440
Moderate	14 (45.9)	9 (31.8)	
Severe	3 (7.9)	2 (4.8)	

*PAL assessed based on Johansson *et al.*, **Fatty liver grading was based on Savarymattu *et al.* NAFLD=Nonalcoholic fatty liver disease, PAL=Physical activity level

Table 3: Mean \pm SD daily total energy and macronutrient intakes before and after intervention

	Green tea extract (n=35)	Placebo (n=36)	P**
Energy (kcal)			
Before	1986 \pm 435	2038 \pm 595	0.83
After	1962 \pm 375	1989 \pm 561	0.87
MD (95% CI)	-31 (-73.69-12.35)	-40 (-82.08-3.58)	
P*	0.145	0.065	
Carbohydrate (g)			
Before	282.2 \pm 95	259 \pm 95.7	0.093
After	275 \pm 71.2	259 \pm 72.6	0.431
MD (95% CI)	53.7 (129.39-212.15)	0.13 (-49.2--22.04)	
P*	0.02	0.996	
Dietary fiber (g)			
Before	16.3 \pm 6.2	16.7 \pm 16.4	0.234
After	15.14 \pm 4.44	22.3 \pm 18.8	0.221
MD (95% CI)	-2 (-3.5-0.40)	4.7 (-4--11.4)	
P*	0.653	0.285	
Protein (g)			
Before	73.3 \pm 18.5	68.4 \pm 24.6	0.391
After	72.7 \pm 16.9	68.2 \pm 24.8	0.983
MD (95% CI)	-0.6 (-3.9-2.9)	-3 (-2.5-0.9)	
P*	0.752	0.731	
Fat (g)			
Before	65.8 \pm 17.8	60.2 \pm 25.3	0.542
After	51.80 \pm 18.80	58 \pm 22.5	0.07
MD (95% CI)	-3.8 (-5-0.8)	-0.46 (-2.3-1.7)	
P*	0.08	0.735	

*Paired t-test, **Independent t-test. SD=Standard deviation, MD=Mean differences, CI=Confidence interval

a reduction in ALT and AST levels at the end of the study, but it was no significant. ALP levels showed

Table 4: Mean \pm SD biochemical factors before and after intervention

	Green tea extract (n=35)	Placebo (n=36)	P
Weight (kg)			
Before	87.22 \pm 11.75	90.51 \pm 14.88	0.331 ^b
After	83.63 \pm 13.7	88.42 \pm 14.35	0.01 ^c
MD (95% CI)	-4.71 (-4.23--2.97)	-3.26 (-3.81--2.55)	
P ^a	<0.001	<0.001	
BMI (kg/m ²)			
Before	34.45 \pm 4.62	34.21 \pm 5.32	0.577 ^b
After	32.09 \pm 3.82	33.41 \pm 4.24	0.01 ^c
MD (95% CI)	-2.36 (-2.68-2.14)	-0.77 (-2.59--2.13)	
P ^a	<0.001	<0.001	
ALT (IU/L)			
Before	44.59 \pm 22.93	43.62 \pm 23.85	0.679 ^b
After	31.38 \pm 18.43	37.88 \pm 22.92	0.251 ^c
MD (95% CI)	-15.23 (-18.68--7.73)	-7.82 (-49--2.51)	
P ^a	<0.001	0.01	
AST (IU/L)			
Before	30.14 \pm 13.21	29.69 \pm 13.42	0.746 ^b
After	22.89 \pm 9.32	26.62 \pm 12.7	0.291 ^c
MD (95% CI)	-11.91 (-30.04--13.77)	-7.68 (-22.34-15.61)	
P ^a	<0.001	0.07	
ALP (IU/L)			
Before	50.59 \pm 55.34	52.15 \pm 70.25	0.771 ^b
After	41.79 \pm 52.81	43.50 \pm 63.15	0.04 ^c
MD (95% CI)	-31.79 (-43.28--16.30)	-3.65 (-9.46--6.81)	
P ^a	<0.001	<0.001	

^aPaired t-test, ^bIndependent t-test, ^cBased on ANCOVA adjusted for baseline values and carbohydrate intake. ALT=Alanin aminotransferase, AST=Aspartate aminotransferase, ALP=Alkaline phosphatase, MD=Mean differences, CI=Confidence interval, SD=Standard deviation, BMI=Body mass index, ANCOVA=Analysis of covariance

significant reductions in both group after 12 weeks period ($P < 0.001$).

DISCUSSION

NAFLD was identified for the first time in people without alcohol usage experience in 1980.^[21] In fact, this disease includes a wide range of disorders from accumulated lipid in the big vesicular form to accumulated lipid with inflammation, cirrhosis, and hepatic damage.^[22] In fact, when NAFLD appears that fat comprises 5–10% of liver's weight.^[23-25] The prevalence of this is 34–46% in adults, although the prevalence of it is 21.5–31.5% in Iran.^[26-28] There have been no certain treatment for NAFLD so far, but losing weight, controlling metabolic syndromes such as diabetes and hyperlipidemia, taking antioxidant drugs like Vitamin D and those which are sensitive to insulin are recommended.^[29,30] In the present study, the effects of GTE prescription on liver function test were evaluated. The results indicated a decrease in weight, after and before the intervention, in both groups. In Jablonski *et al.*, 2013

study which was done on 53 NAFLD patients, the average weight of patients was more than control group, so that patients had high BMI and 10% weight addition to their idea weight.^[31] In our study before any intervention, both groups were homogenized in terms of BMI and weight so that there was no significant statistical difference. The role of BMI and weight in the fatty liver has been the topic of many studies.^[32,33] In the past, BMI was the more important independent predictor agent of accumulated fat in the liver. Other researchers have accentuated that the peripheral accumulated lipid is a more important predictor agent in comparison with BMI.^[34,35] So according to the significant reduction in BMI and weight effected by taking GTE daily in the intervention group, this drug can be considered as an effective drug and can be prescribed for NAFLD patients. In this present, green tea group showed significant reductions in ALT and AST levels after 12 weeks period ($P < 0.001$). The placebo group showed a reduction in ALT and AST levels at the end of the study, but it was no significant. ALP levels showed significant reductions in both group after 12 weeks period ($P < 0.001$). According to these results, it can be claimed that GTE 500 mg prescribed can be considered as an absolute treatment to improve serum levels of liver enzymes in NAFLD patients. While in the results of Takato *et al.*, in 2013 study that evaluated laboratory parameters, BMI, weight, and histological observed liver after taking GTE 100 mg 3 times each day in 12 weeks, ALT declined in green tea group while AST and ALP levels remained unchanged.^[36] The difference between the results of Sakata's study and ours study can be because of the GTE dosage during the study.

EGCG, the main catechin in green tea is believed to reduce liver oxidation stress. The components of NAFLD have not yet been fully elucidated, but the following steps are considered to be the main mechanism. Free fatty acids are absorbed by the liver through the intestinal tract after a meal and are oxidized by mitochondria and peroxisomes. If fatty acid uptake by hepatocytes increases, fatty acid pools in the liver increase and accumulate in the hepatocytes as acylglycerol, increasing the load on hepatic mitochondria. Fatty acids that are not metabolized by mitochondria undergo ω or β oxidation by microsomes or peroxisomes. If a large quantity of fatty acids continues to be deposited in the liver, accumulation of acylglycerol in the hepatocytes induces oxidative stress that may progress to NAFLD.^[37] It has been shown that catechins promote lipid metabolism in the liver.^[38] Body weight and adiposity were blunted by catechin administration in the obese mouse model C57BL/6J. Increased mRNA expression of acyl-CoA oxidase, one of the peroxisomal β -oxidizing enzymes and medium-chain acyl-CoA dehydrogenase, a mitochondrial β -oxidizing enzyme, was observed in the liver of the catechin administration group. Increased hepatocellular mitochondrial β -oxidation

activity promotes the breakdown of fatty acids and it is thought that it acts as a protective mechanism against NAFLD. Catechins are a natural iron chelator and also serve to influence internal absorption of iron. A controlled study looking at the effects of EGCG on nonheme iron absorption showed that it was decreased by 27% in patients consuming 300 mg EGCG compared with controls consuming placebo.^[39] Reports on NASH patients showed that elevated iron stores, iron absorption in the liver^[40] and serum ALT levels were decreased by bloodletting treatment.^[41] Restricting iron absorption through catechins may therefore be effective treatment for NAFLD.

CONCLUSIONS

Results of this randomized clinical trial shows that GTE supplementation decrease ALT and AST levels after 12 weeks period in patients with NAFLD. According to these results, it can be claimed that GTE prescribed can be considered as an absolute treatment to improve serum levels of Liver enzymes in NAFLD patients. Also Lifestyles, modifications, particularly weight reduction may more improve the condition of this disease.

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