Govaresh/ Vol. 25, No.2, Summer 2020; 141-148.

Original Article

A Pilot Study of Epigallocatechin Gallate Treatment in Patients with Non-alcoholic Fatty Liver

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ABSTRACT

Background:
Many trials studied green tea extract for its flavonoid antioxidant effects on the liver function (liver enzymes) and fatty liver status (serum lipid levels) in patients suffering from non-alcoholic fatty liver disease (NAFLD). Therefore, the effects of pure epigallocatechin gallate (EGCG) was assessed here as the most potent flavonoid of green tea in such patients.

Materials and Methods:
This was a pilot study in which 33 patients with NAFLD were assigned to consume EGCG capsules (390 mg) daily for 12 weeks. Fasting blood sugar, liver enzymes, and lipid profiles were monitored at baseline and at the end of the trial.

Results:
The studied group showed significant decreases in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels at the end of trial ($p < 0.01$). The women showed more significant decreases in these enzymes (ALT: $p < 0.01$, AST: $p < 0.001$). No statistically significant differences were observed in total cholesterol, High-density lipoproteins C (HDL-C), Low-density lipoproteins C (LDL-C) , Very low-density lipoprotein (VLDL), and Triglyceride (TG) levels at the end of the trial ($p > 0.05$).

Conclusion:
According to our results and also recent reports about the positive impact of green tea polyphenol EGCG on liver function and fatty liver status in patients with NAFLD, the daily use of pure EGCG may improve the level of liver enzymes in such patients.

Keywords: Epigallocatechin gallate (EGCG), Flavonoids, Antioxidant, Non-alcoholic fatty liver disease (NAFLD)

please cite this paper as:
Alavinejad SP, Shokati Eshkiki Z, Pourmousa Z, Zaeemzadeh N, Mard SA. A Pilot Study of Epigallocatechin Gallate Treatment in Patients with Non-alcoholic Fatty Liver. *Govaresh* 2020;25:141-148.

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Received: 10 Feb. 2020
Edited: 02 Jun 2020
Accepted: 03 Jun 2020

INTRODUCTION

As the main metabolic organ in humans, the liver's health reflects the general wellbeing of an individual. The activity of this metabolic organ has been influenced by alterations that occurred recently in the lifestyle such as food habits and physical activity rate. Non-alcoholic fatty liver disease (NAFLD) is a type of liver disease with the uppermost importance and prevalence in a range of age groups, which commonly induces primary and chronic hepatic disorders around the world (1).

NAFLD consists of a range of conditions induced by fatty infiltration of the hepatocytes with no considerable

alcohol consumption. Additionally, the fatty liver in patients with NAFLD may develop inflammation, necrosis, and fibrosis of the liver, defined as non-alcoholic steatohepatitis (NASH) (2).

NAFLD can originate from multiple lifestyle-associated parameters, including obesity, diabetes, poor diet, and hyperlipidemia, all of which have been suggested as facilitators of the NAFLD occurrence (3).

The disorder is most commonly diagnosed in cases with changed aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations, and also a routine application is the proof of liver steatosis by ultrasonography (3).

The development of cirrhosis resulting from NAFLD in patients will probably lead to death due to liver-linked complications. Thus, it is vital to develop efficient treatments having minimum fallouts against NAFLD to control the development of this disorder to the final-phase liver disorders. A significant goal for the prevention of NAFLD development is still lipid level modification. Approaches for modifying lipid factors might comprise medications, lifestyle alteration, or using plant-derived supplementations (4).

Green tea harbors a plethora of flavonoids consisting of polyphenolic compounds (flavonoid antioxidants) showing antihypertensive, anti-inflammatory, anti-thrombotic, and metabolic impacts, all of which might contribute to their protecting role (5). The above compounds can promote insulin-resistance by mitigating oxidative stress, enhancing endothelial activity, and/or altering glucose metabolism. As the key flavonoid in green tea, catechin has gained ground for its anti-tumor and anti-arteriosclerotic impacts in recent years (6). With the utmost abundance, epigallocatechin gallate (EGCG) is known to be a phenol having the highest potency and high antioxidant activities, which reduces lipid concentrations in humans and is present in many foods including green tea (7).

Clinical questions on the effectiveness of green tea in human have been answered by multiple randomized controlled trials (RCTs); though, the impact of green tea extract containing high levels of catechins (e.g. EGCG) on humans and its complete mode of action have remained unclear with more or less challenging issues, including contradictory findings and rather small sample sizes (8).

As far as the authors are aware, clinical trials have not so far studied pure EGCG. Green tea extract with high-content flavonoids (e.g. EGCG) has a significant contribution to mitigating oxidative stress and improving steatosis and serum ALT levels in the patients (9). Therefore, this research sought to investigate the impacts of medication with pure EGCG capsules (390 mg), as the major flavonoid of green tea, on the liver activity (liver enzymes) and fatty liver status (lipid profile) in patients with NAFLD.

MATERIALS AND METHODS

Subject selection

Totally, 33 patients (80% male and 20% female) with NAFLD in the age range of 35 65 years participated in the study, which was conducted in a prospective manner.

The research goal was precisely described to the participants, and their written informed consent was collected prior to contribution to the trial. This study received approval from the Ethics Committee of Ahvaz-Jundishapur University of Medical Sciences (Ethics committee number: A/1184). The patients were then recruited according to the inclusion and exclusion criteria.

The entire participants were included, provided that they met the criteria of ultrasonographic liver assessment compatible with NAFLD, chronic measurements of liver enzymes (ALT and AST), and lack of or an insignificant history of alcohol use (< 20 g/day). Exclusion criteria were the presence of severe, acute or chronic diseases (liver, heart, or renal failure), Wilson's disease, hemochromatosis, infectious liver diseases (positive for hepatitis B surface antigens, antihepatitis C virus), autoimmune liver disease, metformin consumption, and antihypertensive medication, contraceptive and estrogen consumption, pregnancy, acute infectious diseases, diseases comprising systemic inflammation, alcohol abuse, and using supplementations concomitantly.

This trial was conducted within 90 days. The patients were provided daily with EGCG capsules (390 mg; Puritan's Pride Co., New York, USA). The participants were called once per week to be reminded about the EGCG capsule intake and were asked to report any possible fallouts of the prescription. During the trial, the subjects were supervised to record any alteration in diet, lifestyle habits, and physical activity levels.

The present research was conducted in southwestern Iran at the Alimentary Tract Research Center, Imam Khomeini Hospital, Ahvaz, Iran, from 2013 to 2014.

Experimental protocol

Blood sampling: Blood samples were collected from all subjects in two phases (at the trial onset and at the end) to quantify their liver enzyme concentrations (ALT, AST), fasting blood sugar (FBS), and plasma levels of lipid profile [Total Cholesterol (TCHOL), High-density lipoproteins C (HDL-C), Low-density lipoproteins C (LDL-C) , Very low-density lipoprotein (VLDL), and Triglyceride (TG)]. In the morning of the sampling days, 10 ml of fasting venous blood was sampled from individual participants and collected in empty tubes by a skilled operator. The blood sera were separated through centrifugation (3,000 RPM, 4 °C, 15 min) and kept frozen (-70 °C) for further assays (BT1500-Biotecnica Instruments).

FBS was examined by the glucose oxidase method (Pars Azmoon kit). The kinetic method was used to measure serum AST and ALT levels as a marker of hepatic cell injury (Pars Azmoon Co., Tehran, Iran). AST normally ranges less than 15 U/L and 18 U/L for women and men, respectively. ALT concentrations of below 17 U/L and 22 U/L were regarded as normal for female and male patients, respectively. Triglycerides (TG), total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were assessed through an enzymatic photometric assay (Pars Azmoon kit).

Personal characteristics: Demographic data, including age, lifestyles (diet, drinking, smoking, and exercise habits), socioeconomic status, literacy level, and medical and drug history, were acquired with a self-managed behavior questionnaire on diet and exercise for individual patients. At the entry step, we requested all studied patients with NAFLD not to change their diet and exercise behavior during the study period. The same examiner documented all anthropometric estimates to reduce errors in the initial and the end of the trial. The participants' height was measured with an accuracy of 0.1 cm (Seca stadiometer, Tehran, Iran). The subjects were weighed on a digital balance with an accuracy close to 0.1 kg (Seca scale, Tehran, Iran). Both height and weight were measured by using standard protocols while subjects were wearing light garments with no shoes. At the initial and the end of the trial, body mass index (BMI) was estimated as weight (kg) divided by squared height (m^2). Body fat content was assessed through the Omron body fat monitor (HBF-306).

Hepatic examination. At the start and end of the trial, the liver was assessed by a single-blinded skillful radiologist using ultrasonography (US; General Electric LOGIQ 400 CL- Using probe 3.5/5 MHz). The grade of hepatic steatosis, defined as the content of hepatocytes with fat droplets phases, was calculated and then graded from I to IV for every participant.

Statistical analyses

Data were statistically analyzed using SPSS software version 17. The normal distribution of all variables was first determined with the Kolmogorov-Smirnov test. Then, the baseline values of individual variables were compared with their end values by the use of the paired t test at a significance level of $p < 0.05$.

RESULTS

Of the total 33 selected patients with NAFLD for participating in this clinical trial, three patients were lost during the follow-up period because of personal reasons. Thus, the study was completed by 30 patients.

Baseline demographic and laboratory information of the patients

The demographics and general characteristics such as the mean age, male and female proportion, body weight, BMI, smoking, and education levels of the 30 patients are summarized in table 1.

Accordingly, the percentages of women and men were 20%, and 80%, respectively, with an average age of 38 years. There were no correlations between the education levels and the incidence of NAFLD among the studied group.

The biochemical characteristics of FBS, liver enzymes and lipid profiles of the 30 participants before and after the study are displayed in table 2 and figures 1-4.

Effects of EGCG on FBS, lipid profile, and liver enzymes

Table 1: Demographic and baseline characteristics of patients with NAFLD

Variable	NAFLD
Age	38.18 ± 2.08
Weight	88.59 ± 2.40
BMI	30.27 ± 0.65
Sex	
Female	20.0%
Male	80.0%
Smoking	
Yes	1 (3.33%)
No	29 (96.77%)
Education level	
Illiterate	4 (13.3%)
Below high school diploma	5 (16.7%)
High school diploma	6 (20.0%)
Academic education	15 (50.0%)

Data are the mean ± SEM and frequency of individuals (percentage).

According to table 2, there were no significant differences in the FBS levels at the baseline and the end of the study period. No statistically significant differences were also observed in total cholesterol, HDL-C, LDL-C, TG, and VLDL levels ($p > 0.05$). As shown in table 2, at the end of the research, serum levels of AST and ALT were significantly lower than baseline values, and patients showed significant reductions in ALT and AST levels ($p < 0.01$, figures 1 and 2).

Our findings also showed a strong correlation between sex and AST and ALT serum level depletion. As shown in figures 3 and 4, women showed more decrease in ALT and AST levels than those of men.

DISCUSSION

NAFLD is a liver disease with a potential progression that, if left uncontrolled, raises the peril of liver cirrhosis and hepatocellular carcinoma. Among NAFLD classes, patients with NASH have a poor prognosis. With regard to pathological results, NASH is similar to alcoholic steatohepatitis. Although not the whole cases of NAFLD develop into liver cirrhosis and cancer, timely diagnosis by medical inspection, ultrasonography, and treatment can avert unfavorable consequences (10).

There is a belief that diet therapy is preferred over medical treatment for patients with NAFLD (10). Contradictory findings have been reported from observational data concerning the impact of green tea on lipid parameters. Recently, it has been shown that green tea drinking, up to 4 cups a day, has no associations with fluctuations in lipid markers (11). However, more than 10 cups a day is linked to declining the levels of total and LDL cholesterol, as well as a rise of HDL cholesterol concentration (12).

In green tea, the main catechin is EGCG, which is thought to mitigate liver oxidative stress. Moreover, catechins have been reported to possess inhibitory impacts on lipase associated with glucose and fat absorption. A decrease in fat absorption in the intestinal tract leads to a decrease in liver fatty acid uptake, which may repress the incidence of NAFLD. Reported research indicated that catechins improve lipid metabolism in the liver as well (13).

In a 12-week interventional trial, Sakata and colleagues (2013) studied green tea catechins in patients with NAFLD (14). High-level catechins in green tea reduced the ratio of liver to spleen size, ALT levels, and oxidative stress indicators, but it had no decreasing effects on BMI and weight while assessment by bioelectrical impedance analysis revealed dropped body fat content (15).

ALT and AST concentrations decreased significantly in patients with NAFLD at the completion of this trial. Accordingly, EGCG prescription can be inferred to be a suitable therapy to elevate serum concentrations of liver enzymes in such patients. Recently, a similar study has reported significantly dropped ALT and AST concentrations in patients with NAFLD as an intervention group treated with green tea (16) as observed in our study as well. Takato and co-workers (2013), however, presented evidence that drinking green tea extract (100 mg) trice a day for 12 weeks led to no effect on AST, but it lowered ALT levels (10). The discrepant findings of various trials can be attributed to the medications' variable purity and doses of EGCG used in patients with NAFLD (16).

The most prominent result of the present research was the discovery of a robust association between sex and declined rates of AST and ALT concentrations following EGCG treatment such that more significant declines in serum ALT and AST concentrations were recorded in women than in men. Such an observation has not been described in the literature hitherto, hence

Table 2: Differences in the total cholesterol, HDL-C, LDL-C, TG, VLDL, ALT and AST levels at the baseline and at the end of the study

Variables	Before	After	*p* value
FBS (mg/dL)	96.3 ± 2.1	95.5 ± 2.1	0.24
TCHOL (mg/dL)	198 ± 7.1	183 ± 6.9	0.18
HDL (mg/dL)	43 ± 1.6	40 ± 7.4	0.19
LDL (mg/dL)	131 ± 1.3	115 ± 5.3	0.09
VLDL (mg/dL)	38 ± 2.0	35 ± 1.8	0.21
TG (mg/dL)	172 ± 10.9	157± 12.0	0.11
ALT (IU/L)	77.1 ± 6.6	53.8 ± 4.7	0.01
AST (IU/L)	45.7 ± 3.4	33.8 ± 2.2	0.01

ata are the mean ± SEM. P values indicate differences between before and after (paired t test).
FBS: Fasting blood sugar, TCHOL: Total cholesterol, HDL: High-density lipoproteins, LDL: Low-density lipoproteins, VLDL: Very low-density lipoprotein, TG: Triglyceride, ALT: Alanine transaminase, AST: Aspartate aminotransferase.

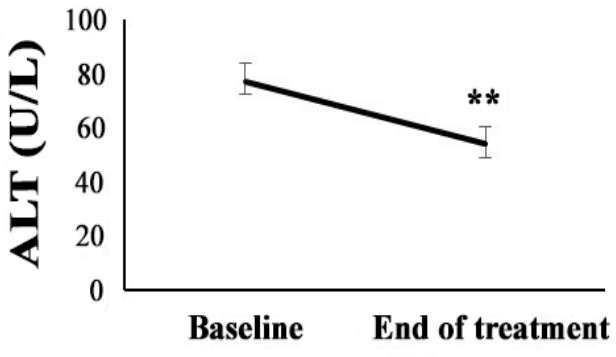

Fig.1: Differences in the ALT levels at baseline and at the end of the study. The values are expressed as mean ± SEM. **p < 0.01.

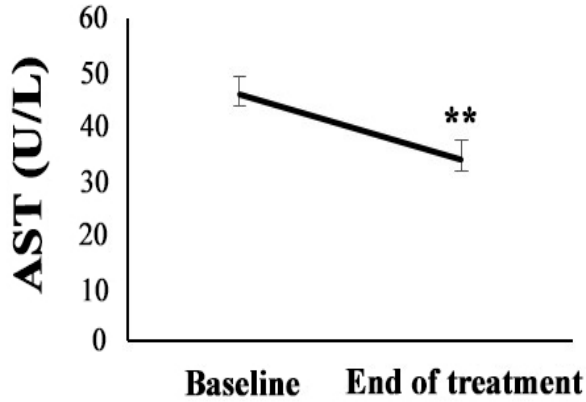

Fig.2: Differences in the AST levels at baseline and at the end of the study. The values are expressed as mean ± SEM. **p < 0.01.

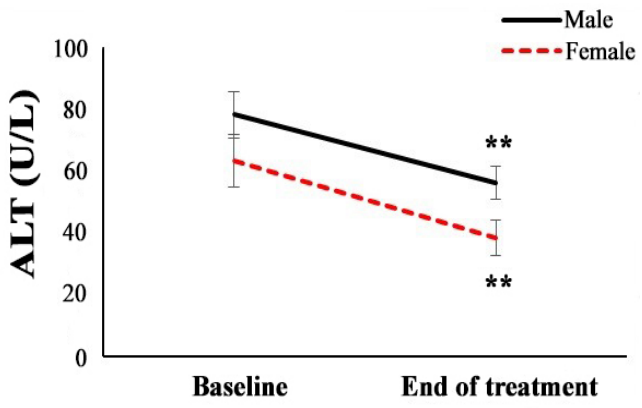

Fig.3: Differences in the ALT levels of men and women at baseline and at the end of the study. The values are expressed as mean ± SEM. **$p < 0.01$ (p values indicate differences between before and after with paired t test).

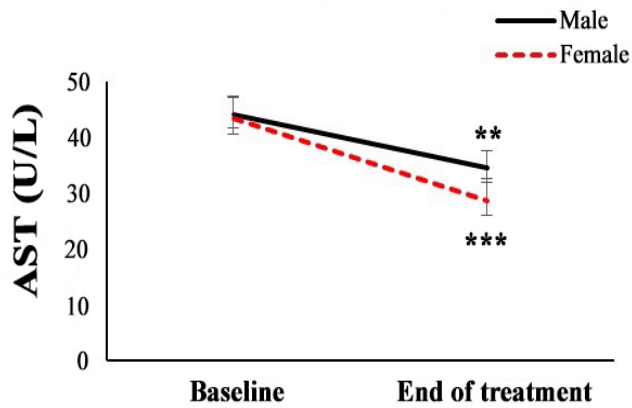

Fig.4: Differences in the AST levels of men and women at baseline and at the end of the study. The values are expressed as mean ± SEM. **$p < 0.01$, ***$p < 0.001$ (p values indicate differences between before and after with paired t test).

this study is the first to report the above relationship. This can probably be explained by the gender characteristics between the subjects.

In general, men present a significantly greater rate of superoxide formation than that of women. Furthermore, men exhibit a lesser antioxidant capability. Thus, an imbalanced reactive oxidative stress (ROS) generation describes the fast senescence process in men. Nevertheless, age-related enlarged oxidative stress does not occur in adult women, thus, indicating that accelerated senescence in men is accompanied by metabolic alterations during men's sexual maturity. The reproductive strategy in men is sustained by fluctuations in metabolism and also ROS generation. ROS probably has a significant contribution to forming life-history determinants (17).

Associations between physiological fluctuations and life history sexual dimorphism (men were active, whereas women stayed sedentary in their burrows) were studied in earlier investigations; hence, it appears reasonable that basal metabolic rate is greater in men than in women (18).

Apparently, human and animal sex dimorphism is largely controlled by sex hormones. As demonstrated in recent research, the activities of antioxidant enzymes in adult men and women were reported to be controlled by such sex steroids as estrogen, while progesterone led to a lesser impact (19). Consequently, sex dimorphism may underlie the dissimilarities between men and women, including differing metabolic rates of antioxidants, which can result in the finding observed in the present trial. It can, therefore, be concluded that the association found between sex and declines of AST and ALT levels following EGCG (antioxidant) treatment was a result of sexual dissimilarities between men and women.

Catechin seemingly influences iron absorption internally and is actually a natural iron chelator. According to a recently reported evaluation, the impacts of EGCG (a type of catechin) on non-hem iron absorption resulted in a 27% drop in the intervention subjects who received 300 mg of EGCG in comparison with the control (placebo) individuals (20).

As observed previously, iron storage and absorption increased in the liver of patients with NASH (21), and serum ALT levels decreased in subjects treated by bloodletting (22). Hence, dropped iron absorption by catechins, in particular EGCG, can be an efficacious limiting cure in patients with NAFLD.

EGCG intake through reduced lipid peroxidation and restored glutathione (GSH) concentrations led to improved steatosis, and inflammation in rats received a full-fat diet (23). A number of laboratory examinations have established the capability of EGCG in the inhibition of stimulating hepatic stellate cells (24). Recently, research has revealed that intraperitoneal EGCG can diminish liver fibrosis by inhibition of NFkB, Akt, and TGF/SMAD signaling and suppression of oxidative stress in a murine model of NASH (25). Repression of LDL oxidation by improving endothelium-bound extracellular superoxide dismutase (EC-SOD) function, a key antioxidative enzyme in the vasculature, is a second mode of action by which green tea catechins restricted liver steatosis (26).

Another outcome of our investigation is that the comparative serum levels of lipid profiles (total cholesterol, HDL-C LDL, VLDL, and TG) were not significantly different at the start and completion of the trial. In this respect, only one experiment by Maron and colleagues (27) disclosed significantly declined total and LDL cholesterol concentrations. In fact, most of the literature represented that epigallocatechin gallate did not significantly affect total and LDL cholesterol levels. An exclusion is a study by Maron and co-workers who evaluated other investigations individually and concluded that concentrations of the lipid parameters (total and LDL cholesterol) stayed unchanged, indicating that this sole trial had no significant skewness on global observations. It is noteworthy that Maron and co-workers used a green tea product supplemented with a black tea polyphenol (theaflavin), which possibly influenced the reported considerable drops of total and LDL cholesterol concentrations (28). Moreover, recently published research indicate that consumption of sour tea and green tea thrice a day for 4 weeks has differently affected specific lipoproteins in patients. The diet elevated HDL-C levels and insulin endurance with type 2 diabetes mellitus (29).

Most glucose metabolic pathways and lipid metabolism in the liver, as well as the hepatic glucose output and lipid synthesis, are under the control of insulin. Accordingly, the homeostasis of glucose

and TG concentrations quickly reflect fluctuations in hepatic sensitivity to insulin. Insulin endurance is an essential pathophysiologic mechanism of NAFLD, diabetes, obesity, dyslipidemia, and hypertension (29).

Similar to previous trials, our results demonstrated that the FBS measures were not significantly different at the starting point and after 12 weeks, which is in line with others reporting no impact of green tea extract on FBS (30).

Totally, the strength of our study is the evaluation of hepato-effects of EGCG in human subjects, and on the other hand, the weakness of the study, as a pilot study, is the small sample size.

CONCLUSION

Based on literature concerning the positive impact of pure EGCG on the liver function and fatty liver status in patients with NAFLD, taking pure EGCG capsules containing a greater dose of this flavonoid appears to be more efficacious than consumption of green tea even more than 10 cups a day.

Based on our observations and other recent reports about the positive impact of EGCG on liver function in patients with NAFLD, the daily use of pure EGCG may improve the level of liver enzymes, particularly in women with NAFLD. Even so, no statistical impacts were observed at concentrations of total, HDL-C, and LDL cholesterol, or triglyceride.

The status of such patients may be further improved through modifications of their lifestyles. Forthcoming trials need to target the best dosage and time span of EGCG. Besides, further research is required to specify other advantages of EGCG.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Ahvaz-Jundishapur University of Medical Sciences for financial support.

CONFLICT OF INTEREST

The authors have declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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