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ORIGINAL CONTRIBUTION

Efficacy and safety of fermented garlic extract on hepatic function in adults with elevated serum gamma-glutamyl transpeptidase levels: a double-blind, randomized, placebo-controlled trial

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Abstract
Purpose Alcoholic liver disease or non-alcoholic fatty liver disease/non-alcoholic steatohepatitis are well-known risk factors for liver fibrosis or cirrhosis and hepatocellular carcinoma; it is a major global health concern, but there are few effective and safe management options. Therefore, we aimed to investigate the effects of fermented garlic extracts (FGEs) on hepatic function in adults with mild hepatic dysfunction without underlying hepatic disease.
Methods In this double-blind, randomized, placebo-controlled study, seventy-five adults with elevated serum gamma-glutamyl transpeptidase (GGT) levels were included in a FGE-administered group ($n = 36$) or a placebo group ($n = 39$), and received either two sachets/day containing FGEs or placebo over a 12-week period. Primary endpoint was the change in serum GGT levels. Data were analysed using a generalized linear mixed effects model.
Results Significant group × time interactions for serum levels of GGT ($F = 3.98$, $P = 0.022$) and alanine aminotransferase (ALT; $F = 3.28$, $P = 0.043$) were observed with an improvement in levels of GGT ($P = 0.066$) and ALT ($P = 0.014$) in the FGE group compared to that reported for the placebo group at the 12-week visits. There was no intergroup difference in the prevalence of adverse events.
Conclusions Intake of FGEs improved serum GGT and ALT levels in adults with mildly elevated serum GGT level without reported adverse side effects. FGEs might be effective and safe management options for mild hepatic dysfunction.

Keywords Alanine aminotransferase · Fatigue · Fermented garlic extract · Gamma-glutamyl transpeptidase · Hepatic dysfunction

In the original publication of the article, the subsection of "Preparation of FGEs" the Sentence 3 and 5 has been published incorrectly; this error has now been corrected.

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Introduction

Chronic metabolic effects of alcohol consumption and other nutritional excess on the liver result in alcoholic liver disease (ALD) or non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH), which are the most common causes of elevation in liver enzyme levels [1] and well-known risk factors for liver fibrosis or cirrhosis and hepatocellular carcinoma (HCC) [2]. Therefore, the burden of liver diseases is a major global health concern. However, the options for management of hepatic injury due to alcohol and other nutritional abuse are limited [3].

Garlic (*Allium sativum* L.) is one of the most popular vegetables worldwide; it is particularly used as a spice in

the Asian and Mediterranean food and has been used in traditional medicine for a long time for its diverse health benefits [4]. It contains various ingredients such as organosulphur compounds including allicin, alliin, S-allyl cysteine (SAC), and S-allyl mercaptocysteine and other bioactive non-sulphur compounds. Numerous studies have reported positive biologic effects of garlic on plasma lipid levels [5], systolic blood pressure [6], inhibition of platelet aggregation and atherosclerosis [7], as well as its antioxidative properties [8] and hepatoprotective effect [9].

Although garlic has health benefits, the pungent smell and flavour of raw garlic make it unpalatable. Therefore, various garlic processing methods and formulations have been developed for reducing its pungent smell and taste while maintaining or improving the contents of raw garlic ingredients. Garlic formulations have shown health benefits similar to those shown by raw garlic. Of them, fermented garlic extracts (FGEs), which are produced by fermentation with *Lactobacillus plantarum*, are not only odourless and quick to produce but also contain higher contents of components such as SAC or cycloalliin than those present in raw garlic [10]. Previous studies have indicated that FGEs possess antidyslipidaemic [11], antidiabetic [12], and antioxidant properties [13]. However, few studies have investigated the effects of FGEs on hepatic dysfunction. In previous studies, we reported that *L. plantarum*-fermented FGEs decreased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in mice with alcohol-induced fatty liver damage [14], and upregulated peroxisome proliferator-activated receptor (PPAR)-α and carnitine palmitoyltransferase-1 mRNA expression as a consequence of ß-oxidation activation in oleic acid-induced steatotic HepG2 cells [15]. To the best knowledge of the authors, no studies have been conducted on the hepatoprotective effects of FGEs in adults with hepatic dysfunction. Therefore, we aimed to investigate the effects of FGEs on hepatic function in adults with mild hepatic dysfunction but without any underlying hepatic disease such as hepatitis B or C virus infection, liver cirrhosis, or HCC.

Materials and methods

Participants and study design

A 12-week, randomized, placebo-controlled trial was conducted to assess the effect of FGEs on the hepatic function of adults with mild hepatic dysfunction. Of the patients who visited one of two medical centres in South Korea, those who had elevated serum gamma-glutamyl transpeptidase (GGT) levels were selected as the subjects of this study. The inclusion criteria were adults aged between 20 and 75 years and fasting serum GGT levels of 76–225 U/L that is no more than three times over the normal upper limit of serum GGT levels (normal range 11–75 U/L). The exclusion criteria were (1) previous or present alcohol or drug abuse, (2) presence of liver cirrhosis or HCC based on a hepatic ultrasonographic scan, (3) AST, ALT, alkaline phosphatase, or lactate dehydrogenase levels (normal range 140–271 U/L), three times more than the normal upper limit, (4) current viral hepatitis B or C, (5) renal dysfunction (serum creatinine levels two times more than the normal upper limit), (6) thyroid dysfunction (thyroid-stimulating hormone levels $\leq$0.1 or $\geq$10 μIU/mL), (7) presence of uncontrolled hypertension or diabetes, or use of antidyslipidaemic agents newly within 3 months, (8) presence of heart disease or neurological or psychiatric disorders, (9) use of a medicine that influences hepatic function, such as valproic acid, tetracycline, allopurinol, phenytoin, sertraline, naproxen, diclofenac, cholagogue or cholelitholytics, and sex or adrenocortical hormones, (10) use of traditional herbs or dietary supplements that can improve hepatic function, and (11) pregnancy or breastfeeding. Of the 107 subjects who attended the screening process, 27 who failed to meet the inclusion/exclusion criteria or cancelled the study participation were excluded. A total of 80 subjects were enrolled in this study. Enrolment began on 18 June 2014, and the last subject visit was conducted on 6 July 2015. All the subjects signed a written informed consent form before the start of the trial. The study protocol was approved by the Institutional Review Board of The Catholic University of Korea (IRB approval number: VC14HDME0050). This study was registered in Clinical Research Information Service (CRiS: https://cris.nih.go.kr/cris/en/) with the identifier code KCT0001181.

Preparation of FGEs

In our previous study, conducted in mice with alcohol-induced fatty liver damage, AST and ALT levels were significantly lower in the experimental group fed a daily dosage of 0.6–0.8 mg/kcal of FGEs, compared to the levels in the placebo group [14]. Assuming that a mean daily energy intake of adults is 2000 kcal, the daily FGE intake would correspond to 1.2–1.6 g; therefore, 1.5 g/day was taken as the FGE dosage for this study. The sachets containing FGEs or placebo used in this study were produced by SK Bioland, Ltd., South Korea. Garlic bulbs were soaked in purified water (1:2 w/v), which was brought to a boil for 3 h at 110 °C. The solution was cooled at 37 °C and fermented with *L. plantarum* BL2 (2 % w/w) in a fermenter for 36 h. After the cultivation, the solutions were sterilized for 30 min at 121 °C, cooled at 50 °C. The fermented solution was filtered using a filter press, and perlite was added to the filtrate. Thereafter, the solution was incrassated by heating and then sterilized for 1 h at 95 °C. Two sachets,

which resulted in a daily dosage of 40 g, contained FGEs (1.5 g), which contains 5.64 mg of cycloalliin, and additive substances (38.5 g; purified water, corn syrup, and *Scutellaria* extracts). The placebo sachet containing only the additive substances with garlic flavour and edible dyes, similar to FGEs, matched the total amount in the sachets containing FGEs.

Intervention

In a double-blinded fashion, the subjects were randomized with an allocation ratio of 1:1 to two groups using computer-generated random numbers by blocks: FGE-administered group and the placebo group. Over a 12-week period, the groups received either 40 g FGE/day or placebo, twice daily after breakfast and dinner (one sachet of 20 g each time). Participants were followed up every 6 weeks (at baseline and at weeks 6 and 12) with clinical evaluations including physical examination, laboratory test, and fatigue scale measurement, and the use of other garlic formulations intake. Adverse events defined as any unfavourable and unintended signs including abnormal laboratory findings, symptoms, or diseases temporally associated with the administration of FGEs or placebo were recorded.

Endpoints and measurements

The primary outcome for the efficacy analysis was the change in serum GGT levels. The secondary outcomes were the changes in other hepatic function markers such as AST levels, ALT levels, and AST/ALT ratio, lipid profiles, and fatigue scale score assessed using Multidimensional Fatigue Scale (MFS) [16]. The measurements were conducted every 6 weeks during the study. Venous blood samples were collected after an overnight fasting period of 8 h. AST, ALT, GGT, total cholesterol, triglyceride, high-density lipoprotein, and low-density lipoprotein cholesterol levels were determined using an auto-analyser (Hitachi 747; Hitachi, Tokyo, Japan). The MFS, which was developed to measure multidimensional aspects of fatigue, is a self-administered instrument restructured by Chang et al. [17] on the basis of Fatigue Assessment Instrument developed by Schwartz et al. [16]. The MFS comprises 19-item scored on a 7-point Likert format and includes three subscales measuring global fatigue severity, dysfunction of daily living activities, and situation-specific fatigue. The MFS asks respondents to report their fatigue status during the final 2-week period. The total MFS and the subscales' scores are obtained by adding the scores of all 19 items and the scores of eight, six, and five items for the subscales of global fatigue severity, dysfunction of daily living activities, and situation-specific fatigue, respectively. Higher values are indicative of a severer degree of fatigue.

Assessment of safety

For the safety analyses, blood pressure, heart rate, and body weight from all of the enrolled subjects were measured at baseline and visit 6 and 12 weeks. Measurement of complete blood count, serum electrolytes levels such as sodium, potassium, chloride, and calcium levels, glucose, total bilirubin, albumin, protein, creatinine, blood urea nitrogen, and uric acid levels, urinalysis, and electrocardiography were performed at baseline and at the 12-week visit. Adverse events were assessed at the 6- and 12-week visits.

Other

Self-reported information on age, sex, marital status, education level, smoking, alcohol consumption, the amount of physical activity, dietary intake, and medical conditions including past or current medical problems and operation history was obtained. Alcohol-related questions included the presence of alcohol drinking during the 6-week period before baseline and at every 6-week visit and the number of standard drinks consumed per week. A standard drink is any drink that contains 12 g of pure alcohol. Standard drink alcohol content of 4.5 vol% in beer, 12 vol% in wine, 6 vol% in Korean traditional makgeolli, 20 vol% in Korean Soju, and 40 vol% in whisky was used in this study. The presence of alcohol consumption was classified as no (no alcoholic drinks consumed within the last 6-week period) or yes. The assessments of physical activity and dietary intake were performed at baseline and at every 6-week visit. Physical activity was assessed as the amount and frequency of physical activity per week, and classified as none, <150 min of exercise per week, or ≥150 min of exercise per week. The nutrition survey was assessed using single 24 h recall and conducted by trained dieticians at baseline and at every 6-week visit and was estimated from the nutrient database of the Korean Nutrition Society [18]. Estimates for daily total energy, carbohydrate, protein, and fat intake were obtained.

Statistical analyses

The sample size was calculated at a total of 80 subjects (each group of 40) with 15 % dropout, obtaining 80 % statistical power and a significance level of 0.05 using two-sided independent *t* test to detect a proper sample size from a difference of 10 with an estimated standard deviation of 11.1 between the groups in serum GGT level or a difference of 4.6 with an estimated standard deviation of 6.8 between the groups in serum AST level [19].

The efficacy analyses in intention-to-treat populations included all randomized subjects who received at least one sachet containing FGEs or placebo and had at least

one post-baseline efficacy assessment. The safety analyses included all randomized subjects who received at least one dose of the sachets containing FGEs or placebo and had at least one safety assessment.

All values were expressed as mean ± SD, numbers (percentages), or as geometric means (95 % confidence intervals) for skewed distributions. Variables with skewed distributions were analysed after logarithmic transformation (log transformation). Demographic characteristics were compared using independent *t* test for continuous variables and the Chi-squared test or Fisher's exact test for dichotomous variables. A generalized linear mixed effects model, assuming log-normal distributions, was used to analyse repeated measurements of serum GGT, AST, and ALT levels, AST/ALT ratio, lipid profiles, and MFS scores from the baseline at each time point between the two groups using intention-to-treat. These models consisted of FGEs- or placebo-administered group effect (group), follow-up effect (time), and FGEs or placebo group-by-follow-up interaction effect (group × time) as fixed effect. In addition, a post hoc analysis for multiple comparisons between groups at weeks 6 and 12 was conducted using a Scheffe analysis. For the safety assessment of FGEs, the Chi-squared test or Fisher's exact test was conducted to evaluate the differences in the prevalence of adverse events between the two groups. Statistical analyses were conducted using SAS (version 9.2, SAS institute, Cary, NC, USA), and $P < 0.05$ was considered statistically significant.

Results

Study population

Eighty participants enrolled in this study were randomized into two groups that were administered FGEs or placebo. Four participants in the FGE group dropped out after enrolment (one because of violation of exclusion criteria; two because of withdrawal of consent; and one was lost to follow-up) and so did one in the placebo group (because of withdrawal of consent). Therefore, 75 participants (36 from the FGE group and 39 from the placebo group) were included in the efficacy analysis. A total of 66 participants except five in the FGE group (three because of compliance rate of 70 % or less and two because of withdrawal of consent) and four in placebo group (two because of withdrawal of consent; one because of compliance rate of 70 % or less; and one because of withdrawal due to adverse events) completed this study (88.0 % completion rate) (Fig. 1). The compliance rates for taking the sachets contained FGEs or placebo were 91.5 and 90.6 % in the FGE and placebo groups, respectively.

Intergroup differences in age, sex, marital status, education level, smoking, level of physical activity, dietary intake, medical condition including the presence of chronic disease or medication history, weight, blood pressure, and fasting glucose levels at baseline were not significant. Total cholesterol levels in the FGE group were lower than those in the placebo group. There were no differences in the intake of other garlic formulations during the study between the two groups. The prevalence of alcohol consumption during the 6-week period before baseline and at the 6-week visit was higher in the placebo group than in the FGE group, but the mean alcohol consumption did not differ between the groups at any visits (Table 1).

Efficacy

Changes in the hepatic function in the FGE and placebo groups are shown in Table 2. A significant group × time interaction for serum levels of GGT ($F = 3.98$, $P = 0.022$), and a decreasing trend of GGT levels in the FGE group than the values in the placebo group compared to baseline, was observed at the 12-week visits ($P = 0.066$). Serum ALT levels were significantly different between the two groups over time ($F = 3.28$, $P = 0.043$) and significantly lower in the FGE group compared to that in the placebo group at the 12-week visit ($P = 0.014$) (Table 2).

Change in fatigue status assessed using the MFS is shown in Table 3. Levels of MFS scales including dysfunction of daily living activities, situation-specific fatigue, and global fatigue severity did not differ between the groups at baseline. On the subscales of situation-specific fatigue and global fatigue severity, there were significant group × time interactions ($F = 3.36$, $P = 0.040$ and $F = 3.50$, $P = 0.035$, respectively). The subscale score of situation-specific fatigue was lower in the FGE group than in the placebo group compared to that at baseline at the 6-week visit ($P = 0.011$), and the global fatigue severity score was lower in the FGE group than in the placebo group at the 12-week visit ($P = 0.018$).

Results of subgroup analyses in participants with alcohol consumption are shown in Tables 4 and 5. Twenty-three and 33 subjects in the FGE and placebo groups, respectively, consumed alcohol. The group × time interaction for serum GGT levels was significant ($F = 3.19$, $P = 0.049$), and the GGT levels were lower in the FGE group than in the placebo group at the 12-week visit compared to that at baseline ($P = 0.015$). With regard to fatigue, the group × time interaction for total MFS and all subscale scores were significant. The total MFS and all subscale scores were lower in the FGE group than in the placebo group at the 12-week visit (Table 5).

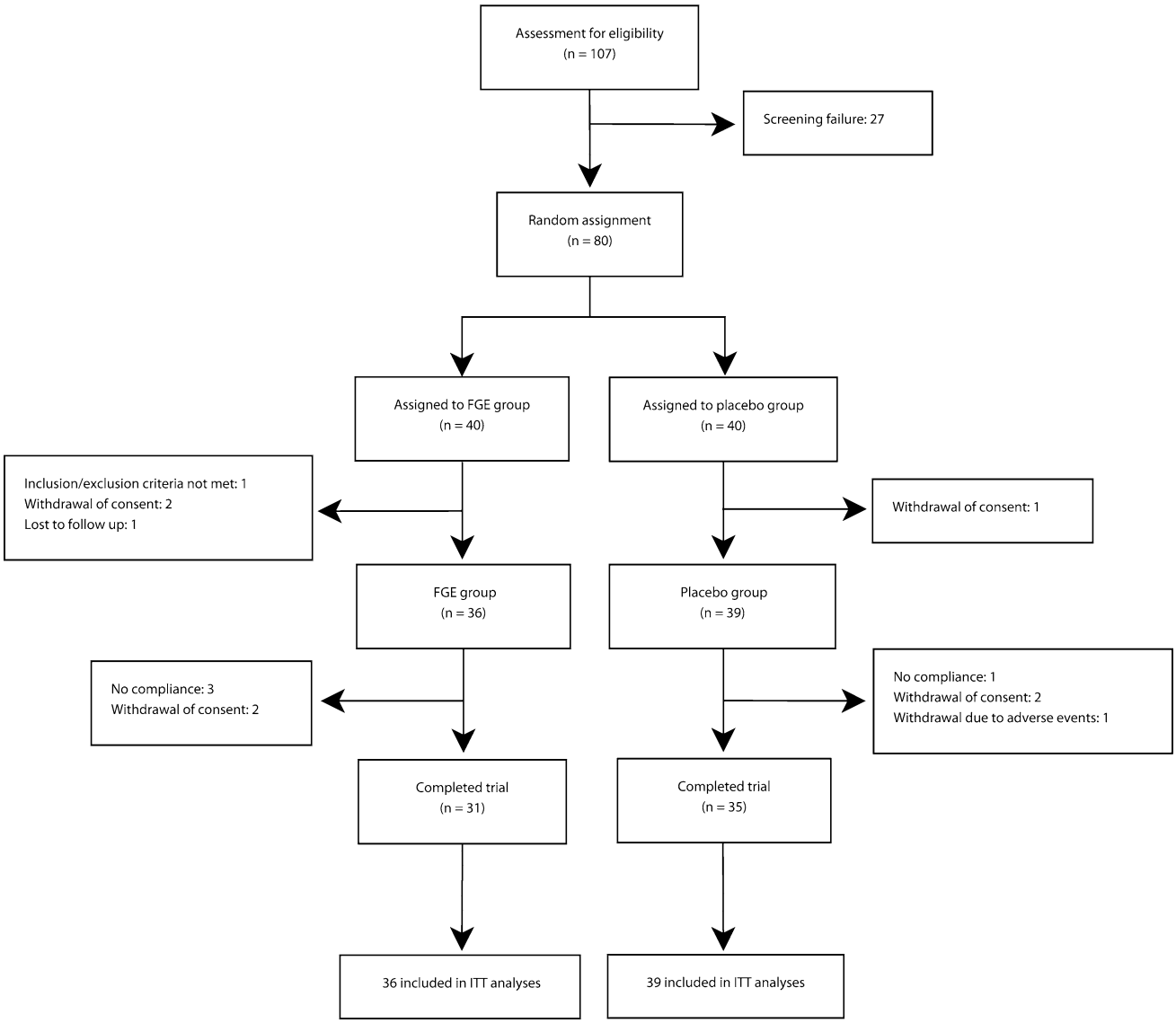

Fig. 1 Flow chart of the study participants

Safety

No significant difference in the prevalence of adverse events was found between the FGE and placebo groups (Table 6). Gastrointestinal problems such as dyspepsia or gastritis were the most frequently reported events in the FGE group. In addition, musculoskeletal problems (arthralgia and myalgia) were detected in two subjects in the FGE group, and cough, dry eye, and colon polypectomy each were reported by one subject in FGEs group. Hepatobiliary disorders such as cholelithiasis, NASH, or abnormal hepatic function were the most commonly reported events in the placebo group. In the placebo group, bronchitis, haemorrhoids, urticaria, oedema, and gout each were reported by one subject. Serious adverse events were reported by two subjects in the placebo group (one with an atrioventricular block on initial electrocardiography and one with a urinary stone), but two subjects completed the study without other problems. In the biochemical and anthropometric measurements conducted for the safety analyses, no significant differences were recorded between the FGE and placebo groups.

Discussion

We investigated the ameliorative effects of FGEs on hepatic dysfunction in Korean adults who had mildly elevated serum GGT levels without underlying hepatic disease. FGEs intake for 12 weeks showed a decreasing trend of

Table 1 Baseline characteristics of the participants and alcohol consumption at baseline and follow-up

	FGEs ($n = 36$)	Placebo ($n = 39$)	P
Sex			
Men	25 (69.4)	30 (76.9)	0.464
Age (years)	54.5 ± 12.2	54.2 ± 9.3	0.888
Marital status			
Currently married	31 (86.1)	34 (87.1)	0.834
Education			
≥College	13 (36.2)	11 (28.2)	0.449
Exercise			
Yes	28 (80.5)	25 (64.1)	0.279
Smoking			
Current	12 (33.3)	18 (46.1)	0.352
High-fat diet			
Yes	22 (61.1)	31 (79.4)	0.164
Chronic diseases			
Yes	35 (97.2)	38 (97.4)	1.000
Medication			
Yes	29 (80.5)	30 (76.9)	0.701
Weight (kg)	70.9 ± 13.3	72.2 ± 12.6	0.673
Systolic blood pressure (mm Hg)	130.3 ± 10.6	130.2 ± 11.7	0.960
Diastolic blood pressure (mm Hg)	75.5 ± 7.9	74.6 ± 8.5	0.623
Fasting glucose (mg/dL)	107.0 ± 21.0	112.4 ± 16.7	0.223
Total cholesterol (mg/dL)	173.8 ± 33.9	190.5 ± 31.6	0.030
LDL cholesterol (mg/dL)	95.2 ± 30.4	105.2 ± 28.2	0.147
HDL cholesterol (mg/dL)	47.6 ± 12.0	52.5 ± 10.4	0.062
Triglycerides (mg/dL)	161.5 ± 33.3	183.5 ± 96.9	0.281
Alcohol consumption			
Baseline	23 (63.8)	33 (84.6)	0.039
6-week visit	23 (63.8)	33 (84.6)	0.039
12-week visit	25 (69.4)	33 (84.6)	0.116
Amount of alcohol consumption			
Baseline	34.6 ± 29.9	29.2 ± 19.3	0.453
6-week visit	30.5 ± 26.0	25.2 ± 17.1	0.395
12-week visit	33.0 ± 35.8	26.4 ± 15.5	0.399

Values are expressed as n (%) for dichotomous variables and as mean ± SD for continuous variables. The amount of alcohol consumption is the number of standard drinks/week

FGEs fermented garlic extracts

Table 2 Changes in hepatic functions at baseline and follow-up

	FGEs ($n = 36$)			Placebo ($n = 39$)			Group × time interaction, F	Group effect, F	Time effect, F
	Baseline	6-week visit	12-week visit	Baseline	6-week visit	12-week visit			
GGT (U/L)	90.9 (79.0–105.6)	85.6 (68.0–107.7)	79.8 (65.3–97.5)	86.4 (75.1–100.4)	83.9 (68.7–101.4)	95.5 (79.0–115.5)	3.98*	0.22	0.71
AST (IU/L)	30.5 (26.0–35.8)	28.5 (24.5–32.7)	27.6 (23.5–32.4)	28.5 (25.0–32.7)	28.5 (25.5–31.8)	30.8 (27.1–34.8)	2.14	0.00	0.44
ALT (IU/L)	35.5 (29.3–42.5)	33.7 (27.9–41.2)	32.1 (26.3–38.8)[a]	30.2 (25.5–35.5)	32.1 (27.6–36.9)	35.5 (30.5–41.2)[a]	3.28*	0.38	0.57
AST/ALT	0.86 (0.76–0.99)	0.84 (0.73–0.97)	0.86 (0.76–0.98)	0.94 (0.84–1.06)	0.89 (0.79–1.00)	0.87 (0.76–0.98)	1.17	0.66	1.31

Enzymatic activities in circulating blood are indicated as values, which are expressed as geometric means (95 % CI)

FGEs fermented garlic extracts, *GGT* gamma-glutamyl transpeptidase, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

* $P < 0.05$

[a] Statistically significant for comparisons between groups at visit 12 week compared with baseline

serum GGT levels and improved ALT levels and scores of global fatigue severity relative to those in the control groups. Additionally, in subjects who consumed alcohol, FGEs intake for 12 weeks improved serum GGT levels, and the scores of MFS and all the subscales relative to those in the control groups.

Various preparations of raw garlic such as heat-treated garlic, aged garlic extract, and garlic-derived constituents

Table 3 Changes in fatigue scale scores at baseline and follow-up

	FGEs ($n = 36$)			Placebo ($n = 39$)			Group × time interaction, F	Group effect, F	Time effect, F
	Baseline	6-week visit	12-week visit	Baseline	6-week visit	12-week visit			
Total MFS score	68.8 ± 28.9	64.6 ± 26.2	63.3 ± 28.4	67.8 ± 22.7	69.1 ± 22.0	72.1 ± 22.9	2.63	0.61	0.43
Subscale 1	21.7 ± 8.9	20.8 ± 8.0	20.1 ± 9.3	22.8 ± 8.3	22.4 ± 7.6	23.2 ± 7.8	1.30	1.31	0.40
Subscale 2	18.7 ± 7.5	17.5 ± 7.2[a]	17.6 ± 7.2	17.2 ± 5.9	18.8 ± 5.7[a]	19.0 ± 6.4	3.36*	0.08	0.14
Subscale 3	28.4 ± 13.5	26.3 ± 12.6	25.5 ± 12.9[b]	27.8 ± 10.8	27.8 ± 10.4	29.9 ± 9.8[b]	3.50*	0.57	0.68

Values are expressed as mean ± SD. Subscale 1, 2, and 3 are dysfunction of daily living activities, situation-specific fatigue, and global fatigue severity, respectively

FGEs fermented garlic extracts, *MFS* Multidimensional Fatigue Scale

* $P < 0.05$

[a] Statistically significant for comparisons between groups at visit 6 week compared with baseline

[b] Statistically significant for comparisons between groups at visit 12 week compared with baseline

have been developed [8] owing to raw garlic's pungency, which limits its intake, and possible toxic effects, such as stomach and liver injury, anaemia, and weight loss [20]. In garlic preparations, the levels of allicin, which is responsible for the pungent smell and taste of raw garlic, are low because of inactivation of alliinase. These preparations contain higher levels of specific organosulphur compounds than those in raw garlic, mainly SAC, alliin, cycloalliin, and S-allylmercaptocysteine, which are stable and have high antioxidant properties [21]. FGEs processed by heat-treating and fermenting garlic bulbs with *L. plantarum* are easy to ingest because they are almost odourless and tasteless [22]. Furthermore, FGEs are produced within a short period of 3 days, whereas the production of an aged garlic extract requires prolonged ageing for up to 20 months [8]. Additionally, microbial fermentation can increase the amount and bioactivity of specific components in foods [23]. *L. plantarum* used in this study is predominantly used in Kimchi, a traditional fermented vegetable food in Korea [24], and the SAC level in FGE was about four times higher than that in unfermented garlic extracts [10].

In this study, elevated GGT levels showed a trend to improve after the intake of FGEs at a 12-week period compared to the levels in the control group. GGT, which is a glycosylated protein embedded in the outer surface of the cell membrane, is expressed in most tissues, with marked expression levels in hepatocytes, biliary epithelium, kidney tubules, and brain capillaries. It provides cysteine, which is the rate-limiting amino acid for glutathione (GSH) de novo synthesis, to cells by breaking down extracellular GSH, the major intracellular antioxidant tripeptide, into its constitutive amino acids, glutamate and cysteinylglycine dipeptide [25]. Thus, GGT plays critical roles in the antioxidant defence system to maintain the homeostasis of cellular GSH, and increased GGT could be an adaptive response to protect against oxidative and toxic stress. Serum GGT level has been widely used as a clinical marker of liver and bile duct dysfunction, diabetes mellitus, and various cancers, and it increases upon exposure to oxidants and substances such as alcohol, drugs, and carcinogens [26]. In previous experimental studies, the hydroxyl radical scavenging activity and the reduction capacity of FGEs were higher than those of the control dried garlic [22]. Further, the superoxide dismutase-like activity, scavenging activity against hydrogen peroxide, and the polyphenol content of FGEs were higher than those of the control garlic extract [13]. A study conducted in rats with alcohol-induced liver damage showed that total GSH and reduced GSH levels in liver tissues of FGE-fed rats were higher than those in control rats [14]. Additionally, SAC, which is abundant in FGEs fermented with *L. plantarum,* has been reported as a powerful antioxidant not only because of its radical-scavenging effects but also because it modulates cell antioxidant defence signalling. It has been reported that SAC inhibits nuclear factor-κB (NF-κB) activation by blocking cytokine production, including that of tumour necrosis factor (TNF)-α, interleukin-1ß, and interferon-γ, and regulating reactive oxygen species-induced transcription [27]. SAC is also known to upregulate PPAR-α, which exerts anti-inflammatory effects through the inhibition of inflammatory cytokines; this suggests that the intake of FGEs in this study can decrease serum ALT levels as a consequence of a modulated immunoactivation, which are used as a marker of mitochondrial damage in hepatocytes. Thus, the antioxidant and inflammatory effect of FGEs could play an important role in the improvement of serum GGT and ALT levels in Korean adults with hepatic dysfunction due to alcohol consumption or other nutritional abuse, but without underlying hepatic disease.

Table 4 Changes in hepatic functions at baseline and follow-up in participants who consumed alcohol

	FGEs ($n = 23$)			Placebo ($n = 33$)			Group × time interaction, F	Group effect, F	Time effect, F
	Baseline	6-week visit	12-week visit	Baseline	6-week visit	12-week visit			
GGT (U/L)	100.4 (83.9–119.1)	86.4 (66.6–112.1)	83.1 (66.0–104.5)[a]	89.1 (76.7–103.5)	91.8 (74.4–112.1)	99.4 (80.6–122.7)[a]	3.19*	0.33	0.42
AST (IU/L)	29.9 (25.5–34.8)	27.9 (23.8–32.7)	28.5 (24.5–33.1)	27.3 (24.0–30.8)	27.9 (24.7–31.5)	31.5 (27.3–36.2)	2.75	0.03	2.01
ALT (IU/L)	33.7 (27.1–42.5)	33.7 (26.8–42.5)	32.7 (26.5–40.8)	28.5 (24.5–33.4)	30.2 (26.0–35.1)	34.4 (29.3–40.8)	2.96	0.38	2.45
AST/ALT	0.88 (0.74–1.05)	0.83 (0.68–1.00)	0.87 (0.73–1.03)	0.95 (0.84–1.07)	0.92 (0.81–1.05)	0.90 (0.79–1.04)	0.34	0.74	1.96

Enzymatic activities in circulating blood are indicated as values, which are expressed as geometric means (95 % CI)

FGEs fermented garlic extracts, *GGT* gamma-glutamyl transpeptidase, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

* $P < 0.05$

[a] Statistically significant for comparisons between groups at visit 12 week compared with baseline

Table 5 Changes in fatigue scale scores at baseline and follow-up in participants who consumed alcohol

	FGEs ($n = 23$)			Placebo ($n = 33$)			Group × time interaction, F	Group effect, F	Time effect, F
	Baseline	6-week visit	12-week visit	Baseline	6-week visit	12-week visit			
Total MFS score	67.5 ± 25.5	60.7 ± 22.3[a]	60.8 ± 25.2[b]	65.5 ± 21.3	68.6 ± 20.9[a]	73.3 ± 21.4[b]	4.18*	1.22	1.95
Subscale 1	21.6 ± 7.8	20.3 ± 7.3	19.2 ± 8.7[b]	22.2 ± 8.0	22.0 ± 7.1	23.7 ± 7.5[b]	4.48*	1.43	0.46
Subscale 2	18.7 ± 6.5	16.5 ± 6.3[a]	17.4 ± 6.8[b]	16.5 ± 5.7	18.8 ± 5.9[a]	19.2 ± 6.1[b]	6.74**	0.15	0.80
Subscale 3	27.2 ± 12.2	23.7 ± 10.1	24.1 ± 11.0[b]	26.8 ± 10.2	27.7 ± 9.7	30.3 ± 9.0[b]	3.47*	1.73	2.11

Values are expressed as mean ± SD. Subscale 1, 2, and 3 are dysfunction of daily living activities, situation-specific fatigue, and global fatigue severity, respectively

FGEs fermented garlic extracts, *MFS* Multidimensional Fatigue Scale

* $P < 0.05$; ** $P < 0.01$

[a] Statistically significant for comparisons between groups at visit 6 week compared with baseline

[b] Statistically significant for comparisons between groups at visit 12 week compared with baseline

Table 6 Adverse events during the study

	FGEs ($n = 40$)	Placebo ($n = 40$)	P
Adverse event	9 (22.5)	11 (27.5)	0.6056
Gastrointestinal disorders	4 (10.0)	1 (2.5)	
Hepatobiliary disorders	–	4 (10.0)	
Cardiac disorders	–	1 (2.5)	
Respiratory disorders	1 (2.5)	1 (2.5)	
Musculoskeletal disorders	2 (5.0)	–	
Urinary disorder	–	1 (2.5)	
Eye disorders	1 (2.5)	–	
Skin disorders	–	1 (2.5)	
Surgical procedures	1 (2.5)	–	
Others	–	2 (5.0)	
Serious adverse event	0 (0)	2 (5.0)	0.4937
Atrioventricular block	–	1 (2.5)	
Urinary stone	–	1 (2.5)	
Withdrawal due to adverse events	0 (0)	1 (2.5)	1.0000
Gastritis	–	1 (2.5)	

Values are expressed as n (%)

Analysis of the subgroup with alcohol consumption revealed that intake of FGEs on improving serum GGT levels in subjects who consume alcohol was significantly effective. Although the mechanism underlying the development of alcohol consumption-induced hepatic cell injuries is not clear, it could be explained by the following factors. In hepatocytes, ethanol is converted to acetaldehyde by a cytosolic enzyme, alcohol dehydrogenase, and a microsomal enzyme cytochrome P450 2E1 (CYP2E1) [28]. Oxidative metabolism of ethanol in the liver produces an excess of reduced nicotinamide adenine dinucleotide, which inhibits the oxidation of fatty acids and triglycerides and promotes lipogenesis [29]. The metabolism of ethanol by CYP2E1 also results in a significant increase in free radicals and subsequently diminishes reduced GSH and other antioxidants, leading to hepatocyte damage [28]. Therefore, the antioxidative effects of FGEs intake could improve the alcohol consumption-induced hepatic cell injuries.

Intake of FGEs improved fatigue scale scores at an overall level. The subjects included in this study were those who had elevated serum GGT levels, a marker of oxidative stress and elevated inflammatory response. Fatigue, as one of the clinical symptoms of acute or chronic infections, inflammatory diseases, and malignancies, is linked to chronic inflammation and oxidative stress [30], and thus, fatigue in the FGE-administered group could be alleviated by the improvements in the oxidative and inflammatory response.

This study has some limitations. Firstly, the sample size was relatively small; however, the study had sufficient statistical power to detect the change of variables. Secondly, we did not consider other indicators for evaluating the effects of FGEs on hepatic function in adults with mild hepatic dysfunction, in addition to laboratory parameters such as serum GGT, AST, and ALT levels. Measurements of additional indicators, including hepatic ultrasonographic scan, abdomen computerized tomography, transient elastography, or liver biopsy, could be more helpful in evaluating the effects of FGEs on hepatic dysfunction.

To our knowledge, this study is the first randomized, placebo-controlled trial to examine the effects of FGE intake on hepatic function in adults with mild hepatic dysfunction. No difference in adverse events was found between the FGE and the control groups, and no participant in FGE-administered group dropped out because of adverse events. The compliance rates for the study material intake were also high, and similar in both groups. From this perspective, the intake of FGEs might be not only effective, but also safe for managing mild hepatic dysfunction in patients without underlying hepatic disease.

In conclusion, hepatic dysfunction, assessed based on levels of hepatic enzymes such as serum GGT and ALT, and fatigue scale scores improved through the intake of FGEs in Korean adults with mildly elevated serum GGT levels, without causing adverse side effects. Our findings suggest that FGEs might be a safe and effective option for the management of oxidative stress and inflammation-induced mild hepatic dysfunction and fatigue in adults without underlying hepatic disease.

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Authors' contribution HNK, SGK, YKR, MKC, and SWS conceived the study and collected the data. HNK and SWS analysed and interpreted the data. HNK, SGK, and SWS drafted the manuscript. All authors supervised writing of the manuscript, provided critical revisions, and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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