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Safety and efficacy of dietary freshwater clam (*Corbicula fluminea*) extract in clinical research

Takao Shimazoe[1*], Daisuke Kobayashi[1], Takehiro Kawashiri[1], Takeshi Chijimatsu[2], Miki Umeki[3], and Satoshi Mochizuki[4]

[1]Department of Clinical Pharmacy and Pharmaceutical Care, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; [2]Sasaki Food Co., Ltd., 276 Sakai, Bungotakada, Oita 879-0615, Japan; [3]Faculty of Food Science & Nutrition, Beppu University, 82 Kitaishigaki, Beppu, Oita 874-8501, Japan; [4]Faculty of Education, Oita University, 700 Dannoharu, Oita 870-1192, Japan

***Corresponding author:** Takao Shimazoe, Department of Clinical Pharmacy and Pharmaceutical Care, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan

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ABSTRACT

Background: The freshwater clam (*Corbicula* spp.) is a popular edible bivalve mollusk that is commonly eaten in East Asia. Freshwater clam extract (FCE) is known to have various effects. For example, it has anti-inflammatory effects and improves cholesterol metabolism. Often used as a folk remedy, FCE might be effective against liver disease and at ameliorating liver damage. These results indicate that FCE has preventative or ameliorating effects against steatosis and mild chronic liver damage. Additionally, FCE has a documented neuroprotective effect, potentially improving sleep quality. However, no clinical research into these topic areas have been carried out.

Objective: No clinical research has been carried out concerning the action of FCE on liver function. In this study, we conducted a clinical trial involving healthy volunteers with relatively high liver test values to determine the influence of FCE on hepatic function. Moreover, no previous studies have described the effects of FCE on sleep. Thus, we also assessed sleep quality after FCE intake using the Oguri-Shirakawa-Azumi (OSA) sleep inventory middle-aged and aged (MA) version

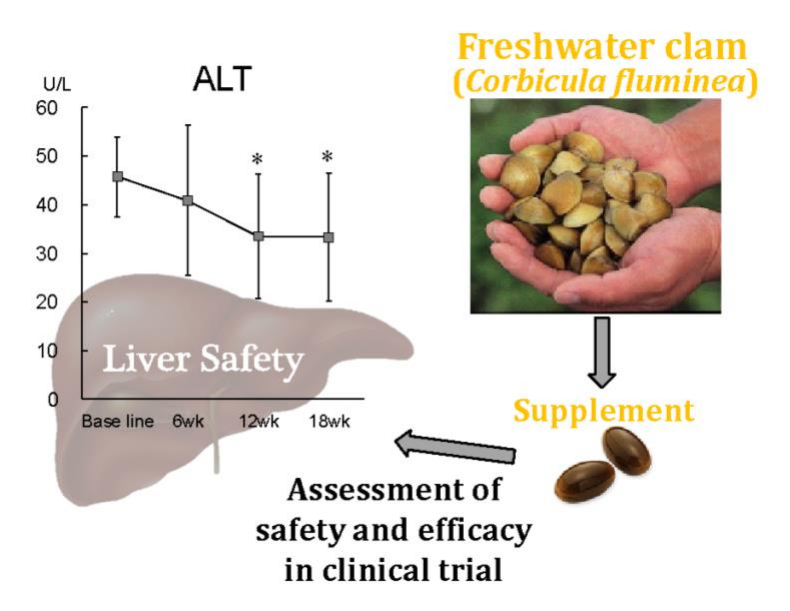
and a Likert scale in a randomized controlled clinical trial.

Methods: We performed a prospective randomized controlled trial to assess safety and the effects of freshwater clam extract. Thirty-four volunteers were analyzed. The subjects ingested 2 placebo softgels, 2 FCE-containing softgels, or 10 FCE-containing softgels. We tried to clarify 2 issues, safety in the liver and quality of sleep. An assessment of the safety of long-term and excessive FCE intake, especially its actions on hepatic function, was performed by administering 10 FCE-containing softgels (5 times the normal dose) to the subjects in the high FCE dose group for 18 weeks. A sleep evaluation comparing the placebo and normal FCE dose groups was also conducted. We conducted a double-blind parallel clinical trial to evaluate the effects of FCE on sleep quality over 12 weeks. The subjects were assigned to 3 groups (the placebo group, the normal FCE dose group, and the high FCE dose group).

Results: Significant reductions in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (γ-GTP) were observed at 12 and 18 weeks after the consumption of a high dose of FCE capsules. The subjects' ferritin levels were significantly reduced after 18 weeks' high-dose FCE intake. Moreover, the consumption of two FCE softgels (normal dose) for 12 weeks resulted in significant better quality in terms of both sleep onset and maintenance compared with that seen after the placebo treatment. FCE intake also resulted in a longer sleep duration than the placebo treatment. The same dose of FCE tended to reduce subjective fatigue. These results suggest that FCE is a safe supplemental food and increases sleep quality.

Conclusions: These results suggest that FCE is a safe supplemental food and increases sleep quality.

Keywords: freshwater clam, hepatotoxicity, randomized study, sleep



INTRODUCTION

The freshwater clam (*Corbicula* spp.) is a popular edible bivalve mollusk that is commonly eaten in East Asia. Freshwater clam extract (FCE) is known to have various effects. For example, it has anti-inflammatory effects and improves cholesterol metabolism [1-13]. We reported that FCE, which is used as a folk remedy, might be effective against liver dysfunction in *in vitro* and *in vivo* [1-2, 4-8]; other studies have found that FCE ameliorates liver damage [9-11]. In a recent study, Lin et al. reported that FCE alleviates non-alcoholic fatty liver disease [12]. In support of this, Yu et al. mentioned that FCE includes hepatoprotective nanoparticles [13]. These results indicate that FCE has preventative or ameliorating effects against steatosis and mild chronic liver damage. On the other hand, Yokomori et al. [14] reported that FCE supplements induced acute cholestasis, suggesting that FCE may not be unequivocally good for hepatic health.

Another potential risk is freshwater clams' relatively high iron content. According to the "STANDARD TABLES OF FOOD COMPOSITION IN JAPAN Seventh Revised", the edible portion of raw freshwater clams contains 8.3 mg of iron per 100 g. Iron is an important nutrient for humans. However, hyperferritinemia and hepatic iron overload were observed in some patients with hepatic disorders [15-17]. Thus, it is hypothesized that excess iron might be one of the causes of hepatic disorders. The influence of FCE on ferritin levels and liver function should be clarified. To date, no clinical research into this topic has been carried out. In this study, we conducted a clinical trial involving healthy volunteers with relatively high liver test values to determine the influence of FCE on hepatic function.

In addition to investigating liver markers, we also assessed sleep quality after FCE intake. FCE is also reported to have some influence on brain function, such as neuroprotective effects [18]. However, no previous studies have described the effects of FCE on sleep, a vital aspect of a healthy lifestyle. Therefore, we also assessed sleep quality after FCE intake using the Oguri-Shirakawa-Azumi (OSA) sleep inventory middle-aged and aged (MA) version [19] and a Likert scale [20] in a randomized controlled clinical trial.

In this study, we aimed to clarify 2 major issues. First, we investigate the safety of FCE as a supplemental food, especially relating to liver health. The second area is whether FCE ameliorates quality of sleep. If we can clarify these factors, we will be able to better inform the utility of FCE as a supplemental food.

METHODS

Subjects and dietary intervention: A prospective randomized controlled trial was conducted at the Medical Corporation Seishinkai Takara Clinic from September 2015 to March 2016. Two hundred and thirty-nine Japanese volunteers over the age of 20 years and habitually drank alcohol agreed to be enrolled in this study. They had relatively low but normal level of liver function. The exclusion criteria were as follows: arterial fibrillation, cardiac arrhythmia, hepatic disorders, renal disorders, cerebrovascular disorders, rheumatism, diabetes, dyslipidemia, hypertension, other chronic diseases, a history of heart failure or cardiac infarction, habitual use of dietary supplements and drugs, being

allergic to medication or clams, pregnant/lactating women, and women who wanted to get pregnant during the trial period. The screening criteria were being negative for hepatitis B virus (HBV) and hepatitis C virus (HCV), having an alanine aminotransferase (ALT) level of approximately 45 IU/L, and being judged by the physicians to be suitable to participate in the trial.

After the screening process, 36 healthy volunteers were enrolled. However, two volunteers dropped out due to personal issues so 34 volunteers were analyzed. A flow diagram of the study is shown in Fig. 1. The subjects were assigned to 3 groups (the placebo group, the normal FCE dose group, and the high FCE dose group). The randomization sequences were created with computer-generated random numbers using Statlight #11 (Yukms, Co. Ltd.). The following stratifications were employed and crossed: i) the mean (± SD) ALT value before the intervention, ii) sex, and iii) age. The key controller conducted the enrollment and allocation procedures. The subjects, physicians, and clinical staff were blinded to the allocation information. The statistical analysts were blinded by keeping the allocation information secret until the primary analyses had finished.

FCE was prepared from *Corbicula fluminea* that were aquafarmed in Taiwan. The approximate composition of the FCE (per 100 g) was as follows: 56.9 g protein, 25.6 g carbohydrates, 4.1 g moisture, 8.8 g crude fat, and 4.6 g ash. In addition, the FCE had a uridine concentration of approximately 0.1%. Each softgel contained 110 mg of FCE. Alpha-tocopherol was also added to the FCE-containing softgels as an antioxidant, as FCE contains some polyunsaturated fatty acids [8]. Therefore, each FCE-containing softgel included approximately 0.1 mg uridine and 10 mg of α-tocopherol. The placebo softgels contained dextrin instead of FCE and α-tocopherol. For masking purposes, a trace amount of caramel coloring was added to the placebo softgels. The subjects ingested 2 placebo softgels (the placebo group, n=11), 2 FCE-containing softgels (the normal FCE dose group, n=12), or 10 FCE-containing softgels (the high FCE dose group, n=11). An assessment of the safety of long-term and excessive FCE intake, especially its effects on hepatic function, was performed by administering 10 FCE-containing softgels (5 times the normal dose) to the subjects in the high FCE dose group for 18 weeks. A sleep evaluation comparing the placebo and normal FCE dose groups was also conducted. In Japan, the guidelines for evaluating the efficacy of food with health claims (advocated by Japanese Ministry of Health, Labor and Welfare) indicate that the minimum intervention period should generally be 12 weeks. Therefore, we conducted a double-blind parallel clinical trial to evaluate the effects of FCE on sleep quality over 12 weeks. The subjects' background information is shown in Table 1. The study was approved by the ethics committee of Medical Corporation Seishinkai Takara Clinic and was conducted in accordance with the Declaration of Helsinki. All of the subjects provided written informed consent.

Hepatic function and other safety parameters: We investigated the clinical data of the enrolled subjects by examining their electronic medical records. Precise information was obtained on the subjects' age, sex, body weight, body mass index (BMI), blood pressure, and

principal liver function test values; i.e., their aspartate aminotransferase (AST), ALT, γ-glutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LD), alkaline phosphatase (ALP), leucine aminopeptidase (LAP), total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), choline esterase (ChE), zinc sulfate turbidity test (ZTT), total protein(T-Protein) and ferritin levels.

Outside of hepatic function, weight, BMI, percentage body fat, systolic blood pressure, diastolic blood pressure, and pulse rate were also assessed as physical parameters. Urinary pH and the levels of urinary protein, urinary glucose, urobilinogen, bilirubin, ketone bodies, and occult blood were evaluated via urinalysis. Furthermore, the white blood cell count, red blood cell count, platelet count, hemoglobin level, hematocrit level, mean corpuscular volume, mean corpuscular hemoglobin level, and leukocyte count were analyzed as hematological parameters. In addition to the principal hepatic function parameters, biomedical blood parameters [alkaline phosphatase, lactate dehydrogenase, leucine aminopeptidase, total bilirubin, direct bilirubin, indirect bilirubin, cholinesterase, total protein, urea nitrogen, creatinine, uric acid, creatine kinase, calcium, sodium, potassium, chloride, inorganic phosphorus, serum iron, serum amylase, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, free fatty acids, blood glucose, hemoglobin A1c, glycoalbumin, and ferritin and the results of the zinc sulphate turbidity test] were also determined.

Subjective sleep evaluation: To assess sleep quality, the OSA sleep inventory MA version (OSA-MA) [21] was used to evaluate the following 5 items: sleepiness on rising, the initiation and maintenance of sleep, dreaming frequency, refreshment, and sleep duration. Subjective symptoms, such as fatigue and sleep-related symptoms, etc., were quantitatively rated on a 6-point (1=strongly disagree to 6=strongly agree) Likert scale [22]. The questionnaire included the following statements:

1. I am physically tired.
2. I am mentally tired.
3. I get drunk easily.
4. I wake up feeling bad the day after drinking.
5. I am satisfied with the sleep I get.
6. I feel bad the day after drinking.
7. I feel stodgy the day after drinking.

Statistical analysis: Data are shown as mean ± SD/95% confidence interval (CI) or median (first quartile-third quartile) values. The significance of the differences within each group were evaluated using Dunnett's test or the paired t-test, and the significance of the differences between groups was analyzed using one-way analysis of variance, the unpaired *t*-test, analysis of covariance, or the Mann-Whitney U-test, as appropriate. *p*-values of <0.05 were considered to be statistically significant.

RESULTS

Subject selection: A flow chart of the study population is shown in Fig. 1. A total of 36 subjects were randomly allocated to 3 groups (the placebo, normal FCE dose and high FCE dose groups). Two subjects dropped out due to personal issues. Therefore, 34 volunteers were statistically analyzed (Fig. 1)

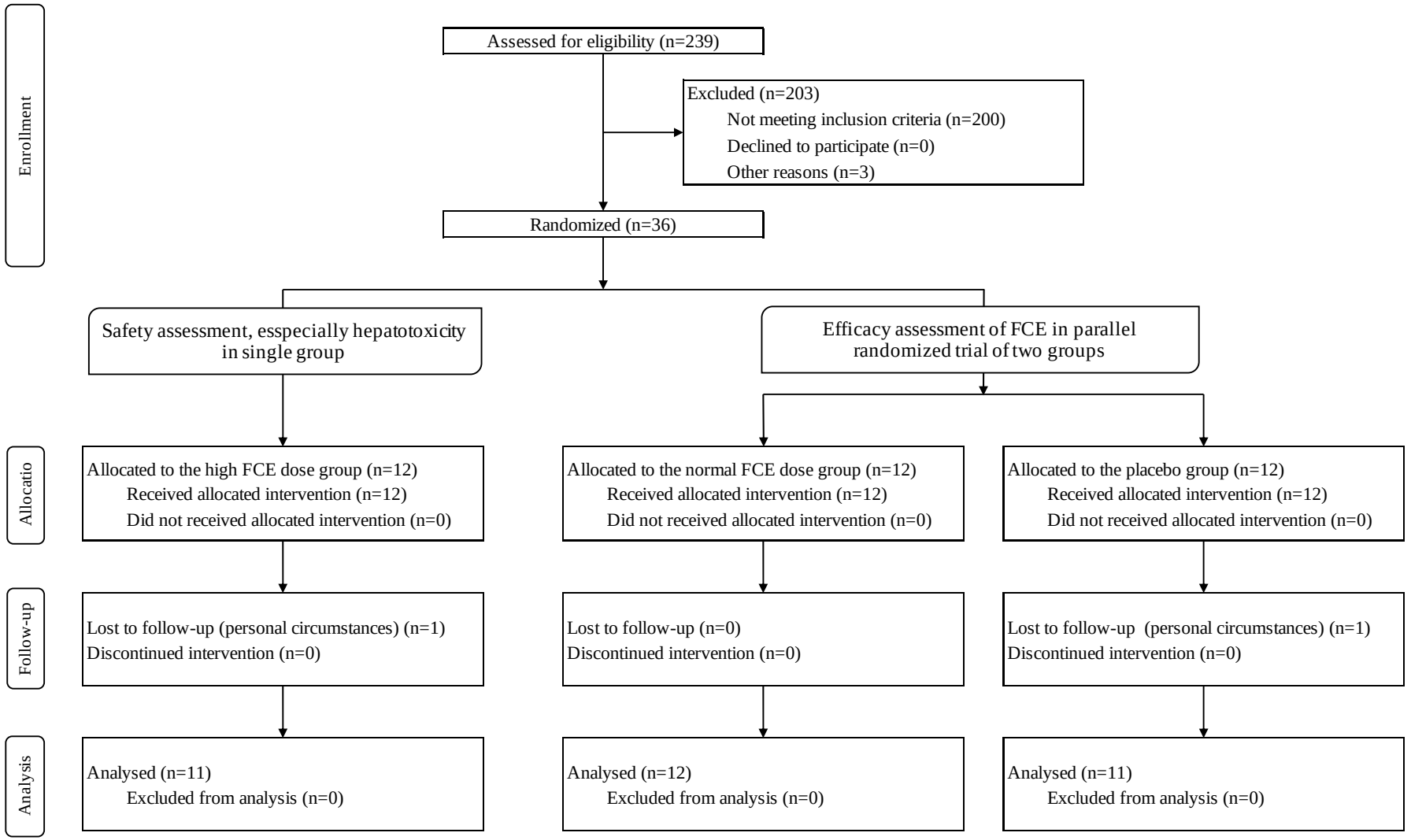


Figure 1. Flow diagram of the study**.** The subjects' physical condition at the baseline (before the intervention) did not differ among the 3 groups (Table 1), and all of the subjects were negative for hepatitis B virus and hepatitis C virus.

Table 1: Baseline characteristics.

Variable	FCE		Placebo group (*n* = 11)	ANOVA
	High dose group (*n* = 11)	Normal dose group (*n* = 12)		
Age, *years*	45.4±12.2	46.6±6.4	47.2±7.4	N.S.
Gender, *male/female, n*	9/2	10/2	9/2	N.S.
Height, *cm*	167±6	171±6	168±5	N.S.
Weight, *kg*	72.2±11.3	82.5±11.7	73.7±16.5	N.S.
BMI, kg/m^2	25.7±2.9	28.1±4.0	26.0±5.2	N.S.
Systolic blood pressure, *mmHg*	133±13	130±12	126±9	N.S.
Diastolic blood pressure, *mmHg*	87±13	88±9	82±9	N.S.
Heart rate, *bpm*	76.6±13.6	77.9±10.6	74.2±11.5	N.S.
AST, *U/L*	30.4±9.0	28.9±6.0	29.3±4.7	N.S.
ALT, *U/L*	45.7±8.2	45.7±10.7	44.9±7.8	N.S.

Data are expressed as mean ± SD values, except for the data related to gender. The significance of the differences within each group was evaluated by one-way analysis of variance. N.S., not significant; AST, aspartate transaminase; ALT, alanine transaminase

Hepatic function and other safety parameters: We tried various statistical assessments from various angles. We selected only representative data. The subjects in the high FCE dose group exhibited significant reduction of AST, ALT, γ-GTP and ferritin levels after 6, 12 or 18 weeks compared with baseline (Fig. 2). There were no significant changes in ALP, LD, LAP, T-BIL, D-Bil, I-Bil, ChE, ZTT, T-Protein levels throughout the experimental period (Fig. 2). Furthermore, high-dose FCE intake for 18 weeks caused significant fall of ferritin levels (Fig. 2). Therefore, the hepatic safety of FCE softgels ingestion is strongly supported. Moreover, there were no problematic changes in any of the other examined parameters in the normal or high FCE dose groups (data not shown). Then, we showed biomedical and hematological blood parameters in high-dose group (Table 2). All of the other data are not significant. Because we could not identify any adverse effects related to FCE intake, the hepatic and overall safety of consuming FCE-containing softgels was corroborated.

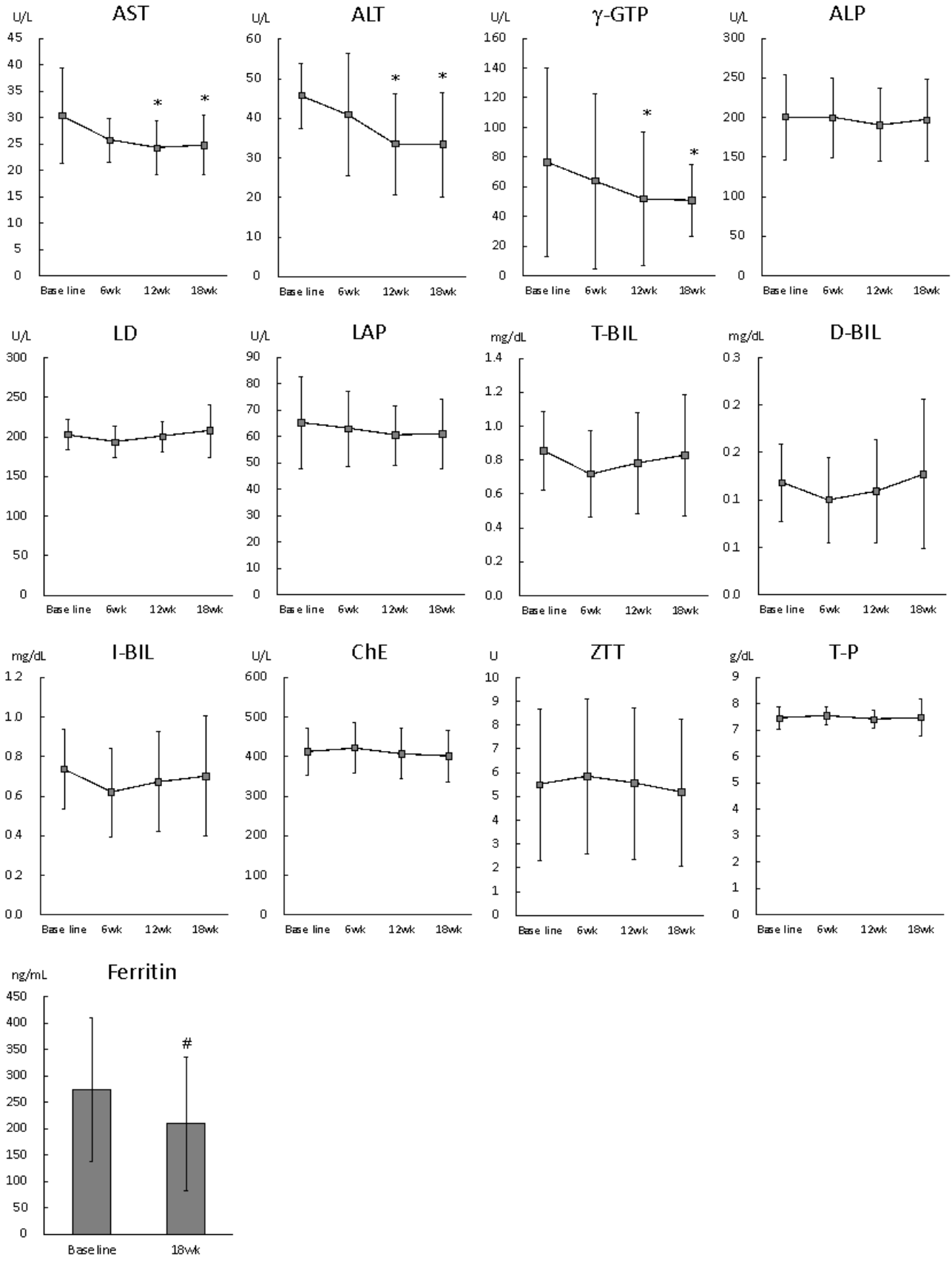


Figure 2. Change of hepatic function observed in an 18-week treatment with daily FCE

Table 2: Hematological and biomedical blood parameters in FCE high-dose group

	Base line	6wk	*p*-value	12wk	*p*-value	18wk	*p*-value
Hematological parameters							
White blood cell count, */μL*	5663.6±898.8	5509.1±1003.1	0.979	5772.7±1054.9	0.992	6218.2±1929.6	0.547
Red blood cell count, *×10⁴/μL*	497.5±39.8	506.9±43.3	0.144	500.2±45.8	0.896	500.8±47.2	0.826
Platelet count, *×10⁴/mL*	25.7±6.2	27.3±6.5	0.503	26.6±6.4	0.849	24.7±7.5	0.741
Hemoglobin, *g/dL*	15.2±1.1	15.5±1.2	0.393	15.2±1.2	1.000	15.1±1.4	0.973
Hematocrit, *%*	45.9±3.1	47.3±3.1	0.081	46.3±3.3	0.847	46.5±4.3	0.660
Mean corpuscular volume, *fL*	92.4±4.0	93.7±4.1	0.096	93.0±3.7	0.622	93.1±4.1	0.524
Mean corpuscular hemoglobin, *pg*	30.6±1.3	30.5±1.1	0.957	30.4±1.1	0.658	30.3±1.3	0.172
Biomedical parameters							
Urea nitrogen, *mg/dL*	12.0±2.8	12.6±2.7	0.737	12.3±2.3	0.936	11.7±2.5	0.944
Creatinine, *mg/dL*	0.8±0.1	0.8±0.1	0.547	0.8±0.1	0.994	0.8±0.1	0.581
Uric acid, *mg/dL*	6.4±1.0	6.1±0.7	0.479	6.1±0.8	0.602	6.2±0.8	0.728
Creatine kinase, *U/L*	148.4±84.6	143.4±79.6	0.996	166.8±105.3	0.866	160.9±88.7	0.951
Calcium, *mg/dL*	9.8±0.4	9.6±0.3	0.431	9.7±0.4	0.956	9.8±0.5	0.852
Sodium, *mEq/L*	141.0±1.0	139.7±1.1	0.018	141.0±1.7	1.000	141.9±1.3	0.114
Potassium, *mEq/L*	4.1±0.3	4.5±0.4	0.003	4.1±0.3	0.748	3.8±0.4	0.005
Chloride, *mEq/L*	101.8±1.6	101.9±1.7	0.997	101.3±2	0.682	100.8±3.1	0.234
Inorganic phosphorus, *mg/dL*	3.1±0.6	2.9±0.3	0.377	3.4±0.6	0.282	3.8±0.9	0.010
Serum iron, *μg/dL*	117.5±41.1	106.5±30.4	0.829	90.7±26	0.236	111.1±45.0	0.957
Serum amylase, *U/L*	77.4±14.8	83.2±16.8	0.319	75.9±11.2	0.965	74.3±12.4	0.763
Total cholesterol, *mg/dL*	230.4±46.4	249.6±42.0	0.029	230.6±42.0	1.000	228.9±46.7	0.994
HDL-cholesterol, *mg/dL*	59.5±18.0	62.0±16.2	0.486	59.4±17.6	0.999	60.3±19.1	0.969
LDL-cholesterol, *mg/dL*	139.6±44.9	153.5±40.1	0.188	142.9±42.4	0.950	137.5±49.5	0.986
Triglycerides, *mg/dL*	149.7±86.3	155.3±74.8	0.989	125.0±54.2	0.553	157.2±90.2	0.975
Free fatty acids, *mEq/dL*	0.7±0.2	0.5±0.1	0.053	0.6±0.2	0.701	0.7±0.2	0.972
Glucose, *mg/dL*	86.3±10.2	86.6±11.0	0.989	86.8±10.7	0.965	87.1±11.3	0.897
Hemoglobin A1c, *%*	5.6±0.4	5.6±0.4	0.475	5.6±0.4	0.354	5.7±0.4	0.020
Glycoalbumin, *%*	12.4±1.3	12.3±1.3	0.512	12.5±1.4	0.723	12.6±1.4	0.512

Data are expressed as mean ± SD values. The significance of the differences from the baseline was evaluated by Dunnett's test.

Blood aspartate transaminase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LD), leucine aminopeptidase (LAP), total bilirubin (T-BIL), direct bilirubin (T-BIL), indirect bilirubin (I-BIL), choline esterase (ChE), zinc sulfate turbidity test (ZTT), total protein(T-P) activity levels and blood ferritin concentrations (liver function biomarkers) detected before and after the ingestion of freshwater clam extract-containing softgels in healthy adults (n=11). Data are shown as mean ± SD values. * indicates a significant difference ($p < 0.05$) from the baseline according to Dunnett's test. # indicates a significant difference ($p < 0.01$) from the baseline according to the paired t-test.

Quality of sleep and subjective symptoms: The results of the OSA-MA are shown in Figure 3. The FCE normal dose group displayed significantly higher sleep initiation and maintenance scores than the placebo group at 12 weeks (p-value = 0.031) (Fig. 3C). In addition, the change in the sleep duration score between the baseline and 12 weeks was greater in the normal FCE dose group than in the placebo group, but did not reach statistical significance (normal FCE dose group: 4.6 vs. placebo group: -3.7; p-value=0.077). Sleepiness upon rising, dreaming frequency, length of sleep, and how refreshing the sleep was did not change significantly in the study period. Overall, these results suggest that the ingestion of FCE-containing softgels improved sleep quality.

Then, we evaluated various subjective symptoms using a Likert scale. The score for subjective physical fatigue (I am physically tired) and the change in the score for waking the day after drinking (questionnaire: I wake up feeling bad the day after drinking) between the baseline and 12 weeks tended to be improved by the ingestion of FCE-containing softgels compared with the ingestion of the placebo (Table 3).

Effects of freshwater clam extract (FCE)-containing softgels/placebo softgels on sleep quality as evaluated by the Oguri-Shirakawa-Azumi sleep inventory MA version (OSA-MA) in healthy adults. Data are shown as mean ± 95% CI values. The significance of the differences between the FCE (n=12) and placebo groups (n=11) was evaluated using the Student’s t-test (baseline) or analysis of covariance (ANCOVA) (6 weeks and 12 weeks).

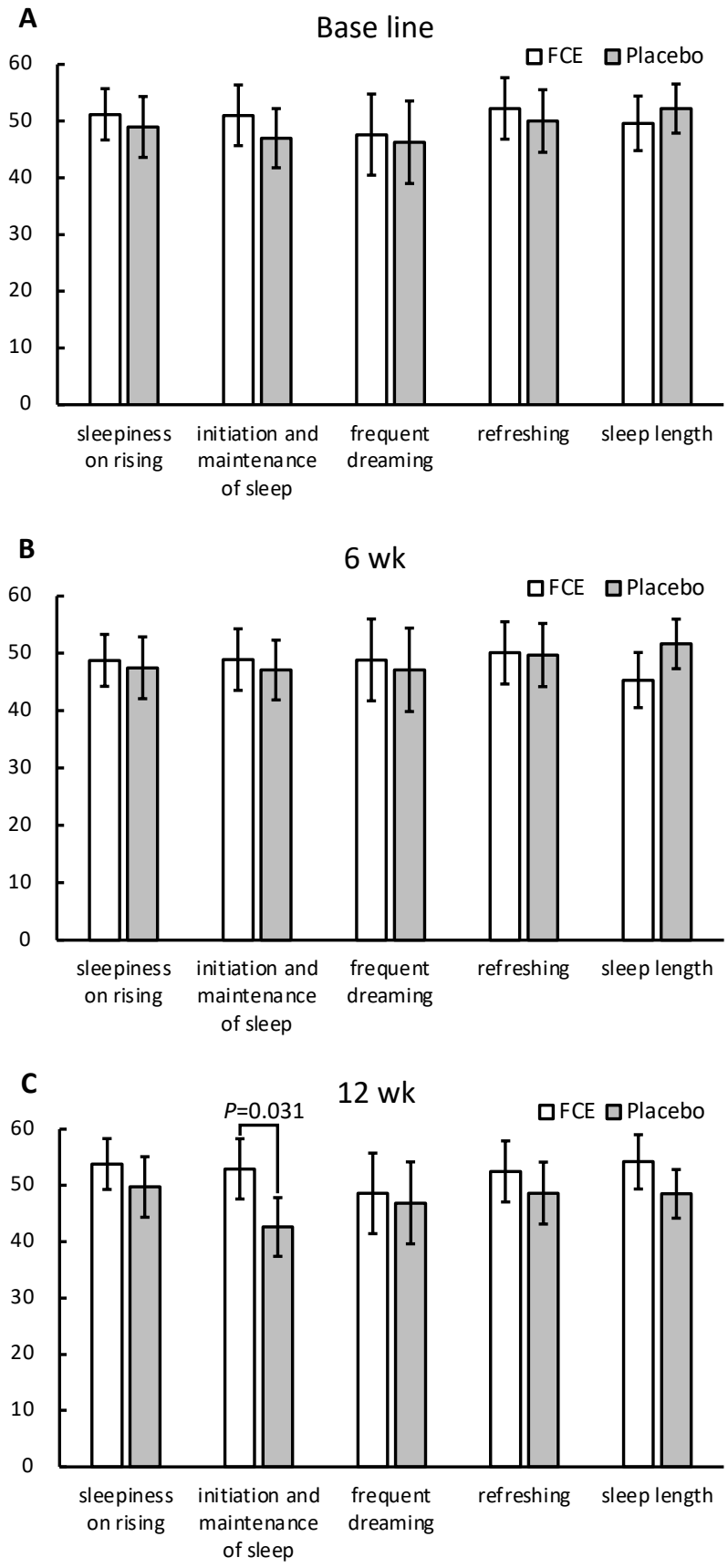


Figure 3. Sleep score in the Oguri-Shirakawa-Azumi sleep inventory.

Table 3: Subjective symptoms rated on a Likert scale.

Items	FCE normal dose group (n = 12)		Placebo group (n = 11)		*p*-value *	
	Value after the intervention (12 wk)	Change from baseline	Value after the intervention (12 wk)	Change from baseline	Value	Change
I am physically tired	2.5(2.0-3.0)	0.0(-1.0-0.0)	3.0(2.5 -4.0)	0.0(-1.0 -1.0)	0.097	0.480
I am mentally tired	3.0(2.0-3.3)	0.0(0.0 -1.0)	3.0(2.0 -4.0)	0.0(-1.0 -0.0)	0.810	0.233
I get drunk easily	2.5(1.0-3.3)	-0.5(-1.3 -0.3)	3.0(2.0 -5.0)	0.0(0.0 -0.5)	0.225	0.204
I wake up feeling bad the day after drinking	2.0(2.0-3.0)	-0.5(-1.3 -0.0)	2.0(2.0 -3.5)	0.0(-0.5 -1.5)	0.567	0.097
I am satisfied with the sleep I get	4.0(3.0-4.3)	0.0(-1.3 -0.3)	3.0(2.5 -4.0)	0.0(-0.5 -0.0)	0.430	0.945
I feel bad the day after drinking	2.5(1.0-3.0)	-0.5(-1.3 -0.0)	2.0(2.0 -3.0)	0.0(-1.0 -0.0)	0.690	0.335
I feel stodgy the day after drinking	2.0(1.0-3.0)	-1.0(-2.0 -0.0)	2.0(2.0 -3.0)	0.0(-1.0 -0.5)	0.398	0.291

Data are expressed as median (first quartile-third quartile) values. * Mann-Whitney U test

DISCUSSION

We assessed the safety, especially its influence on hepatic function, and effects, especially its effects on sleep quality, of FCE intake. It has been suggested that hepatic disorders might be caused by hyperferritinemia and hepatic iron overload [15-17]. In general, the serum ferritin level is used as an indicator of iron storage in the liver. In addition, Kell and Pretorius found that it is an important inflammatory marker, making measuring the serum ferritin concentration important in this study [21]. This result demonstrated for the first time that daily FCE intake reduced serum ferritin level in clinical trial. Therefore, we concluded that patients will be able to take FCE without monitoring ferritin level. Moreover, no hepatic disorders occurred after FCE intake, demonstrating that FCE does not have any toxic effects on hepatic function.

Not only is FCE safe, literature suggests that daily FCE intake might improve hepatic function. Indeed, FCE showed hepatoprotective effects in rats with carbon tetrachloride-induced hepatic damage [22]. In addition, we previously demonstrated that FCE suppressed the accumulation of hepatic cholesterol [7, 8]. Consistent with the literature, we observed that daily consumption of 10 FCE softgels (5x normal) lead to significant reductions in the levels of AST, ALT, and γ-GTP, markers of liver damage.

It should be noted that not all literature supports FCE's role in improving hepatic function. In fact, clam extract-induced cholestasis has been reported in a clinical case study [12]. However, other factors might

have contributed to the observed hepatic toxicity. Regardless, our clinical data showed that FCE has positive effects on hepatic function.

FCE's utility may also apply to sleep quality, which no previous studies have described in animals or humans. In our preliminary study, FCE improved sleep quality in mice (unpublished data). Therefore, we conducted a clinical study to assess the effects of FCE on sleep quality. According to the OSA-MA, the normal FCE dose group exhibited significantly higher sleep initiation compared to the control after 12 weeks of supplementation. These results suggest that FCE might improve sleep quality.

We cannot clarify the exact mechanism by which FCE affects hepatic function and sleep because FCE is a natural product that includes various substances. However, FCE is known to include uridine. Uridine has been reported to be effective against hepatic damage [23]. In previous studies, the administration of uridine was found to improve sleep [24] and memory function [25]. Dobolyi et al. [26] showed that uridine has positive effects on the central nervous system. Therefore, uridine might be a key substance in the effects of FCE. Further studies will be necessary to determine the exact mechanism responsible for the effects of FCE.

The greatest limitation of this study is the relatively small cohort size, which may be responsible for a faulty estimation of the magnitude of observed associations. Further clinical trials might be necessary to expand upon our findings.

CONCLUSION

We conducted a clinical trial to assess the safety of FCE in the liver its effects on sleep. We found that daily consumption of 10 FCE softgels (5x normal) lead to significant reductions in the levels of AST, ALT, and γ-GTP, markers of liver damage. Two FCE softgels a day resulted in improved sleep initiation and maintenance compared to the placebo treatment. For the first time, we clarified that FCE improves liver function and sleep and does not have any adverse effects in clinical trial, even in high doses (5x normal).

Abbreviations: ALT: alanine aminotransferase, AST: aspartate aminotransferase, BMI: body mass index, ChE: choline esterase, D-BIL: direct bilirubin, FCE: freshwater clam extract , γ-GTP: γ-glutamyl transpeptidase, HBV: hepatitis B virus, HCV: hepatitis C virus, I-BIL: indirect bilirubin, LD: lactate dehydrogenase, LAP: leucine aminopeptidase, MA: middle-aged and aged, OSA: Oguri-Shirakawa-Azumi, T-BIL: total bilirubin, T-Protein: total protein, ZTT: zinc sulfate turbidity test.

Competing interests: All of the authors do not have any conflict of interest.

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