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Original Article

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A Randomized, Double-Blind, Placebo-Controlled, Multi-Centered Clinical Study to Evaluate the Efficacy and Safety of *Artemisia annua* L. Extract for Improvement of Liver Function

Byoungduck Han[1,2] **Seon-Mee Kim**[2] **Ga Eun Nam**[3] **Seon-Hee Kim**[4] **Su-Jin Park**[5] **Young-Kyu Park**[6] **Hyun Wook Baik**[7]

[1]Department of Family Medicine, Sahmyook Medical Center, Seoul 02500, Korea
[2]Department of Family Medicine, Korea University Guro Hospital, Seoul 08308, Korea
[3]Department of Family Medicine, Korea University Anam Hospital, Seoul 02841, Korea
[4]Sungkyun Biotech, Co., Ltd., Suwon 16648, Korea
[5]G&P Bioscience Co. LTD., Goyang 10326, Korea
[6]Department of Family Medicine, Bundang Jesaeng Hospital, Seongnam 13590, Korea
[7]Division of Gastroenterology & Clinical Nutrition, Department of Internal Medicine, DMC Bundang Jesaeng Hospital, Seongnam 13590, Korea

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Correspondence to
Hyun Wook Baik
Division of Gastroenterology & Clinical Nutrition, Department of Internal Medicine, DMC Bundang Jesaeng Hospital, 20 Seohyeon-ro 180beon-gil, Bundang-gu, Seongnam 13590, Korea.
E-mail: hwbaik@gmail.com

ORCID iDs
Byoungduck Han
https://orcid.org/0000-0003-2830-1174
Seon-Mee Kim
https://orcid.org/0000-0002-1957-2928
Ga Eun Nam
https://orcid.org/0000-0002-6739-9904
Seon-Hee Kim
https://orcid.org/0000-0003-4079-2594
Su-Jin Park
https://orcid.org/0000-0002-2693-3664
Young-Kyu Park
https://orcid.org/0000-0001-6095-7136
Hyun Wook Baik
https://orcid.org/0000-0003-1410-0232

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) has the potential to develop into hepatic steatosis and progress to terminal liver diseases such as cirrhosis and hepatocellular carcinoma. This human clinical study was aimed to demonstrate that SPB-201 (powdered-water extract of *Artemisia annua*) can improve liver function in subjects with non-alcoholic liver dysfunction at mild to moderate levels. A decrease of 271% in aspartate aminotransferase (AST) level and a significant decrease of 334% in alanine aminotransferase (ALT) level was observed in the test group as compared to the control group at the 4 weeks follow-up. In addition, after 8 weeks, decreases of 199% in AST level and 216% in ALT level were reported in the test group as compared to the control group. These results confirmed that SPB-201 intake significantly enhanced liver function and health. Moreover, the Multidimensional Fatigue Scale score of the test group decreased but that of the control group increased, implicating that SPB-201 also eliminated overall fatigue. No significant adverse events were observed among all subjects during the study. Taken together, our clinical study confirmed the excellent efficacy and safety of SPB-201 in liver function improvement, showing the possibility of SPB-201 as a functional food to restore liver dysfunction and treat liver diseases.

Keywords: *Artemisia annua*; NAFLD; Clinical study; Fatigue; Functional food

INTRODUCTION

According to statistics from the Centers for Disease Control and Prevention (CDC), 4.5 million American adults have liver disease, accounting for 1.8% of the total population in 2018 [1]. The death rate among patients with chronic liver disease increased from 21.9 per 100,000 population in 2007 to 24.9 in 2016, increasing by 1.3% per year for men and 2.5% for

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Conflict of Interest

The authors declare that they have no competing interests.

women. The death rate from among patients with non-alcoholic fatty liver disease (NAFLD), in particular, which accounts for 34.7% of chronic liver diseases, increased by 2.1% per year from 7.3 per 100,000 population in 2007 to 9.0 in 2006 [2]. As of 2015, out of the 143.9 million people with major chronic diseases in South Korea, 1.49 million have liver disease [3]. NAFLD has not received much attention due to its relatively mild clinical prognosis, however, it has the potential to develop into hepatic steatosis and progress to terminal liver disease such as cirrhosis and hepatocellular carcinoma [4,5].

In a cohort study on 420 NAFLD patients with a mean follow-up of 7.6 years, cirrhosis occurred in 3% cases [6]. Liver cirrhosis occurred in 0.9% of 109 NAFLD patients in 16.7-year study and in 1.2% of 170 NAFLD patients in 20.4-year study [7, 8]. In Korea, the prevalence of NAFLD is rapidly increasing reaching 27.3%, which is believed to be largely attributed with stress and Westernization of diet [9-11].

Depending on the underlying cause, liver diseases can be classified as viral-induced, alcoholic, toxic (drug-induced), fat-accumulating, autoimmune, and metabolic liver diseases [12]. Since liver cells may gradually be destroyed and lose more than half of their functional capacities without any specific symptoms, it is therefore important to maintain normal liver function and prevent liver failure [13]. Although lifestyle correction and medication are the current standard treatments for liver diseases, there is a concern about the safety of long-term administration of drugs, and further studies on their effectiveness are needed.

Artemisia annua L., one of the most widely distributed wormwood in Korea, has shown excellent pharmacological efficacy against a wide range of diseases such as osteoarthritis, rheumatoid arthritis, obesity, hyperglycemia, hyperlipidemia and cardiovascular disease, and further demonstrated anti-gastritis, and anti-ulcer effects, all of which are derived from its strong anti-inflammatory and antioxidant activities [14-24].

In a previous study using a high fat diet (HFD)-induced fatty liver mouse model, *A. annua* extract maintained normal liver weight in non-induced fatty liver mice and showed histologically similar patterns to that in non-induced normal liver mice, but not in the HFD-induced group. In addition, the *A. annua* extract improved the blood levels of aspartate transaminase (AST), alanine transaminase (ALT), and hepatic triglyceride (TG) by 40% in both the HFD-induced and the non-treated group and reduced the total blood cholesterol (TC) to near normal levels. It was also confirmed that expression of phosphorylation of AMP-activated protein kinase (p-AMPK) and acetyl-CoA carboxylase (p-ACC) in the liver tissues of mice treated with *A. annua* extract increased to near-normal levels, while the expressions of fatty acid synthase (FAS) and sterol regulatory element binding protein (SREBP)-1c decreased. Similar results were shown in in vitro studies of lipid metabolic gene expressions using the HpeG2 cell line [25-27]. In addition, in the lipopolysaccharide (LPS)/D-galactosamine (GalN)-induced liver failure mouse model, *A. annua* extract reduced AST and ALT levels, and protected the hepatocyte from fatal liver damage. In the in vitro test using the Raw264.7 cell line, it was proved that hepato-protective effect of *A. annua* extract came through the reduction of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and nitric oxide (NO) [28].

Therefore, this human clinical study was conducted to verify the effectiveness and safety of 8-week SPB-201 (powdered, water extract of *A. annua* [WEAA]) therapy in improving the liver function of subjects with liver function impairments.

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MATERIALS AND METHODS

This human clinical study was conducted in accordance with the International Council for Harmonization of Good Clinical Practice (ICH GCP) guidelines, Korean GCP (KGCP), and the Helsinki Declaration. In addition to the standard guidelines of the Ministry of Food and Drug Safety and the pre-approved protocol of the Institutional Review Board (IRB) of Seoul Sahmyook Medical Center (SYMC IRB 1808) and Bundang Jesaeng Hospital (IMCN IRB 18-04). IRB was approved for launching on August 8, 2018 and for completion in SYMC IRB 1808 and IMCN IRB 18-04 respectively in May 14, and June 1, 2020. All participating subjects (or legal representatives) were provided sufficient explanation prior to written consent. All study procedures and data were checked by regular monitoring.

Subjects and study design

In this human clinical study, 170 participants were screened based on the election/exclusion criteria, through which 74 subjects were eliminated, leaving a total of 96 subjects to be randomly assigned into the two groups (n = 48 in each group). At the end of the study, 4 subjects from the test group dropped out due to violation of the selection/exclusion criteria, and 1 withdrew consent while 3 subjects from the control group withdrew consent, and 1 dropped out due to loss of follow-up. A total of 87 subjects completed the study (test group, n = 43 and control group, n = 44) (**Figure 1**).

Per protocol (PP) analysis set was defined as a subject who completed the study and does not have any serious violations (selection/exclusion criteria violation, etc.) that affected study result. Three cases of selection criteria violation (ALT > 120 U/L; test group, n = 1 and control group, n = 2) and 5 cases of inadequate compliance (test group, n = 4 and control group, n =

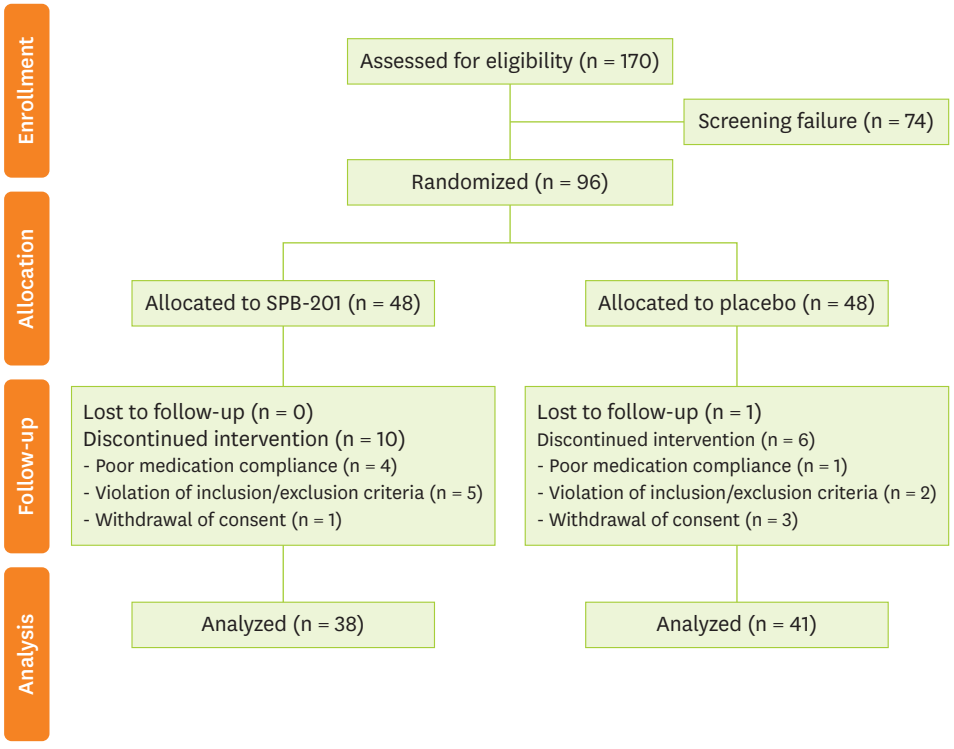

Figure 1. Flowchart of participants, recruitment, and randomization of the study.

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1) were excluded. The PP analysis set eventually composed of 79 subjects (test group, n = 38 and control group, n = 41) (**Figure 1**).

The subjects selected were adult men and women with borderline and mild liver dysfunction with blood AST or ALT in the range of 45–120 U/L. Selected subjects who voluntarily participated in the study, were randomly assigned to either the test group or control group according to the registration order, and were evaluated in parallel for 8 weeks (56 days). Those who were treated for alcohol use or induced disorder, those with liver dysfunction such as cirrhosis, liver cancer, or hepatitis, and who took drugs that affect liver function were excluded. The test group consumed *A. annua* hot water extract powder (SPB-201, 686 mg/2 tablets/day [solids based on 480 mg/day] twice a day in the morning and evening). Placebos, which contain crystallin cellulose instead of SPB-201, were provided to the control group in the same manner.

Sample collection and laboratory assessment

Follow-ups were carried out at weeks 4 and 8 and involved the following tests; 1) Occurrence of adverse events, 2) Changes in combination therapy, 3) Physical examinations (clinical evaluation for abnormality in respiratory, cardiovascular, digestive, endocrine, reproductive, musculoskeletal, and nervous systems), 4) Vital signs (pulse, blood pressure, and bio-impedance) 5) Physical measurements (height and weight), 6) Clinical pathology tests (hematological examination, hemochemical test, urine test, etc.), 7) Electrocardiography (ECG), 8) Multidimensional Fatigue Scale (MFS, survey for evaluation in global fatigue severity, daily dysfunctional fatigue, and situation-specific fatigue), 9) Drinking and smoking habits (survey on the average, amount and frequency of the week during the previous month), 10) Dietary habits (to maintain the usual diet and dietary intake and not to regularly consume mugwort-related foods), 11) Compliance, 12) Excising habits (survey for the average, duration and frequency of the week during the previous month).

The main outcome parameter of the clinical study is the amount and rate of change in blood AST and ALT after 4 and 8 weeks of ingestion of the substances. In addition, the MFS and blood cholesterol were confirmed as parameters for efficacy.

Statistical analysis

Statistically analyses were performed using SAS® (Version 9.4, SAS Institute, Cary, NC, USA). Descriptive statistics (number of subjects, average, standard deviation, minimum and maximum values) were presented for patient's demographics and clinical characteristics. For numerical data comparing the amount of change before and after the test, a 2-sample t-test or Wilcoxon rank-sum test was performed depending on normality satisfaction. Categorical data were presented as frequency and ratio, and the χ^2 test or Fisher's exact test was conducted for independence verification. Significant probability values are presented for all results.

In the analysis of safety endpoints, all subjects who consumed the test substance or placebo after randomization were included. For the analysis of the efficacy evaluation variables, only the test subjects who have completed the study without any serious violations that would affect study results (such as, selection/exclusion criteria violation, etc.) were included. The data obtained were presented as mean and standard deviation, and were considered significant when $p < 0.05$.

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RESULTS

This human clinical study was designed to demonstrate that SPB-201 improves liver function of subjects with non-alcoholic liver dysfunction at mild to moderate levels. Compounding factors were assessed by comparing all patient demographic and clinical characteristics (**Table 1**). The test group included 26 men (68.42%) and 12 women (31.58%), while the control group included 32 men (78.05%) and 9 women (21.95%). There were no statistically significant differences in the gender between the groups. The average age was 48.00 ± 12.63 years and 49.20 ± 12.94 years in the test and control groups, respectively, with no statistically significant differences. Body weight was 76.02 ± 16.15 kg and 77.45 ± 15.93 kg, respectively, with no significant difference between groups. The body mass index (BMI) (calculation using body weight in kilograms [kg] and height in meters squared [m^2]) was also not significantly different between the test and control groups, averaging at 26.65 ± 3.87 kg/m^2 and 27.53 ± 4.26 kg/m^2, respectively. In addition, no statistically significant differences were observed for exercise, smoking status, smoking amount, smoking period, notification method, among others. The comparability between the randomly assigned groups was therefore confirmed prior to initiation of administration of SPB-201 and the placebo.

In the analysis of the AST level changes at the 4-week follow-up, mean AST level in the test group decreased by 12.16 ± 12.41 U/L ($p < 0.0001$), while that in the control group decreased by 4.49 ± 11.27 U/L ($p = 0.0147$). Significant differences were demonstrated between the 2 groups ($p = 0.0045$ and $p = 0.0421$; 2 sample t-test and Wilcoxon rank sum test, respectively). At the 8-week follow-up, these reduced by 11.39 ± 13.58 U/L ($p < 0.0001$) in the test group, and by 5.71 ± 12.71 U/L ($p = 0.0017$) in the control group. Although there was no statistically significant

Table 1. Baseline participants demographics and clinical characteristics

Clinical characteristics	SPB-201 (n = 38)	Placebo (n = 41)	Total (n = 79)	p value
Sex				0.3331†
Male	26 (68.42)	32 (78.05)	58 (73.42)	
Female	12 (31.58)	9 (21.95)	21 (26.58)	
Age (yr)				0.6794*
Mean ± SD	48.00 ± 12.63	49.20 ± 12.94	48.62 ± 12.73	
Min–Max	20.00–67.00	19.00–73.00	19.00–73.00	
Weight (kg)				0.6881*
Mean ± SD	76.02 ± 16.15	77.45 ± 15.93	76.77 ± 16.05	
Min–Max	48.1–117.4	53.2–144.5	48.1–144.5	
BMI (kg/m^2)				0.3499*
Mean ± SD	26.65 ± 3.87	27.53 ± 4.26	27.10 ± 4.10	
Min–Max	18.24–34.79	22.48–47.73	18.24–47.73	
Exercise				0.8229‡
No	14 (36.84)	18 (43.90)	32 (40.51)	
1–2 times/week	9 (23.68)	12 (29.27)	21 (26.58)	
3–4 times/week	10 (26.32)	8 (19.51)	18 (22.78)	
5–6 times/week	3 (7.89)	2 (4.88)	5 (6.33)	
Everyday	2 (5.26)	1 (2.44)	3 (3.80)	
Smoking				0.2203†
No	22 (57.89)	20 (48.78)	42 (53.16)	
Ex-smoker (over a year)	3 (7.89)	9 (21.95)	12 (15.19)	
Smoker	13 (34.21)	12 (29.27)	25 (31.65)	
HFD				0.8381†
No	4 (10.53)	6 (14.63)	10 (12.66)	
1–2 times/week	27 (71.05)	27 (65.85)	54 (68.35)	
3 or more/week	7 (18.42)	8 (19.51)	15 (18.99)	

SD, standard deviation; BMI, body mass index; HFD, high fat diet.
*The p value by 2 sample t-test; †p value by χ^2 test; ‡p value by Fisher's exact test.

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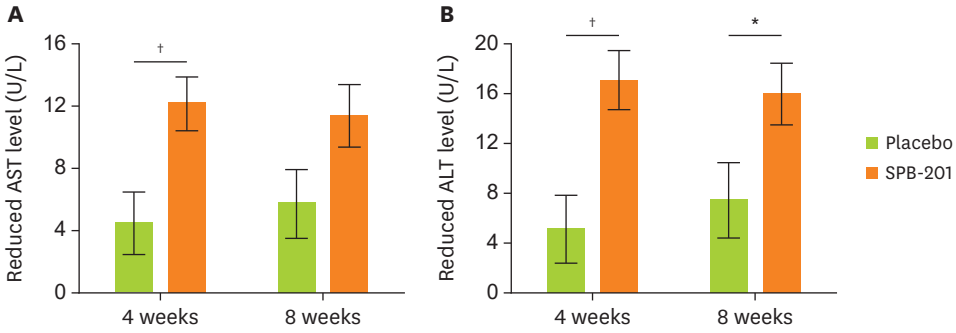

Figure 2. Changes in serum concentrations of AST and ALT in SPB-201-intake subjects.
Serum levels of AST and ALT were measured before (week 0) randomization of the subjects. All subjects were followed-up at 4 weeks and 8 weeks after the study. (A) The trend of decrease in AST levels from weeks 0, 4, to 8. (B) The trend of decrease in ALT levels from weeks 0, 4, or 8. The white bar represents the reduction trend of the placebo group, while the dark bar represents the that of the SPB-201 test group. Data are expressed as mean ± standard deviation (placebo group, n = 41 and test group, n = 38).
AST, aspartate transaminase; ALT, alanine transaminase.
*$p < 0.05$, †$p < 0.01$.

difference between the 2 groups ($p = 0.0626$), the decrease in AST levels in the test group was more prominent than that in the control group, and showed a clear improvement (**Figure 2A**).

In the analysis of ALT level changes at the 4-week follow-up, mean ALT level in the test group decreased by 17.05 ± 16.89 U/L ($p < 0.0001$) and that in the control group decreased by 5.10 ± 15.22 U/L ($p = 0.0381$). Significant differences were observed between the two groups ($p = 0.0014$ and $p = 0.0030$; 2 sample t-test and Wilcoxon rank sum test, respectively). At the 8-week follow-up, the change in mean ALT level in the test group decreased by 15.97 ± 18.82 U/L ($p < 0.0001$), and that in the control group decreased by 7.41 ± 15.90 U/L ($p = 0.0048$). There was a statistically significant difference in ALT level changes between the two groups at both the 4-week ($p = 0.0014$) and 8-week ($p = 0.0317$) follow-ups, reflecting remarkable improvement in liver function with SPB-201 (**Figure 2B**).

In the AST change rate analysis, after 4 weeks of substance intake, the test group showed a decrease by 0.23 ± 0.21 U/L and the control group showed a decrease by 0.11 ± 0.25 U/L, with statistically significant differences between the 2 groups ($p = 0.0144$). After 8-week follow-up, statistically significant differences were not observed between the 2 groups, but the AST change rate of the test group decreased by 0.21 ± 0.22 U/L, and that of the control group decreased by 0.13 ± 0.26 U/L. The AST reduction rate of the test group was dramatically reduced and maintained throughout the study (**Supplementary Figure 1**).

After 4-week follow-up from substance uptake, the ALT change rate decreased in the test group by 0.26 ± 0.24 U/L and in the control group decreased by 0.09 ± 0.28 U/L, showing statistically significant differences between the two groups ($p = 0.0063$). No statistically significant differences were not observed between the groups after 8-week follow-up, but ALT reduction rate of the test group was 0.24 ± 0.26 U/L, and that of the control group was 0.14 ± 0.30 U/L clearly indicating that the ALT improvement rate of the test group was superior to that of the control group (**Supplementary Figure 2**).

With regard to the total change in the MFS, the test group showed improvement by 0.63 ± 12.21 and 0.71 ± 15.40 points after 4 and 8-week follow-up, respectively, while the control group showed deterioration by 2.05 ± 11.92 and 2.12 ± 11.99 points. The differences in

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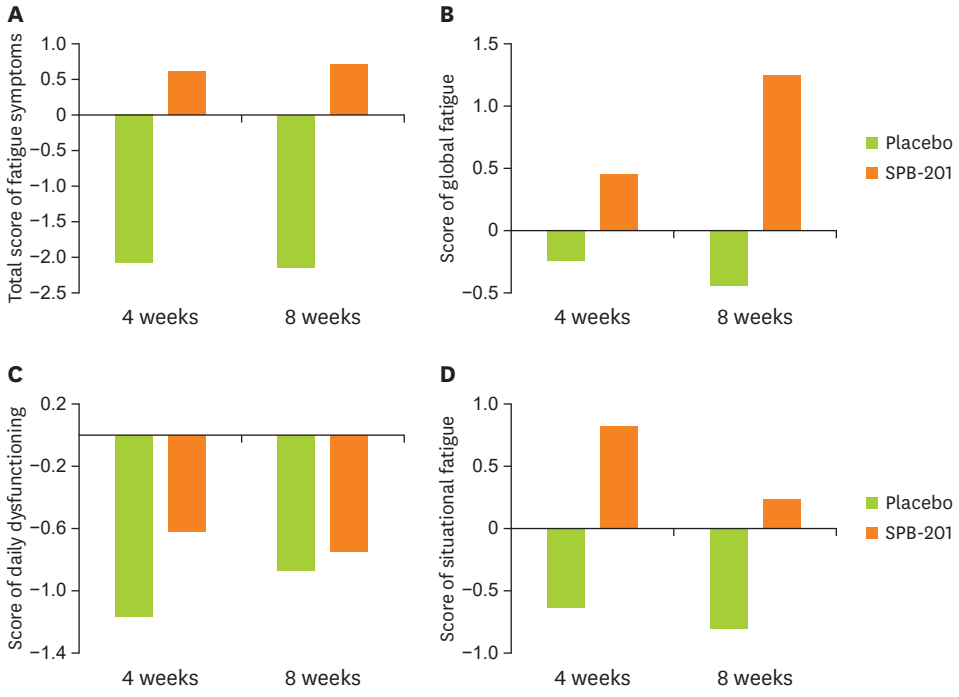

Figure 3. Changes in MFS in the test group.
All registered subjects were followed-up before (week 0) and after substance intake (weeks 4 and 8) for evaluation of the MFS. (A) The total score of MFS consisted of (B) global fatigue severity, (C) daily dysfunctional fatigue, and (D) situation-specific fatigue. The change of each fatigue scores from weeks 0 to 4 or 8 of each group are indicated. The white bar represents the change in the placebo group, while the dark bar represents that in the SPB-201 test group. Data are expressed as mean ± standard deviation (placebo group, n = 41 and test group, n = 38).
MFS, Multidimensional Fatigue Scale.

MFS between the 2 groups increased with time (**Figure 3A**). Regarding changes in daily life dysfunctional fatigue, after 4-week and 8-week follow-up, the test group showed improvement of 0.45 ± 5.59 and 1.24 ± 5.63 points, respectively, while the control group showed exacerbation by 0.24 ± 5.90 and 0.44 ± 5.77 points respectively (**Figure 3B**). Global fatigue severity improved by 0.82 ± 6.66 points and 0.24 ± 7.87 points, respectively, in the test group and showed a tendency to increase the fatigue rate by 0.63 ± 6.15 points and 0.80 ± 6.06 points respectively in the control group (**Figure 3D**). After both time points, the overall fatigue level of the test group decreased but that of the control group increased, demonstrating that SPB-201 intake eliminated overall fatigue.

The total cholesterol of the test group ingesting SPB-201 decreased by 20% compared to the control group. In particular, the low-density lipoprotein-cholesterol of the test group decreased by 6.03 ± 23.14 mg/dL after 8 weeks of administration, while the control group increased by 0.02 ± 21.08 mg/dL, but there was no statistical significance between the intake groups (data not shown).

The safety endpoint analysis was conducted on a total of 87 subjects who completed the clinical study (test group, n = 43 and control group, n = 44). The test items were derived from hematologic tests (**Table 2**) and hemochemical tests (**Table 3**). Hematological analyses included white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) level, hematocrit (Hct), mean corpuscular volume (MCV), and platelet, neutrophil, lymphocyte, monocyte, eosinophil, and basophile counts. After 8-week follow-up, Hct of the test group

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Table 2. Change of hematological parameters

Hematological parameters	SPB-201		Placebo		p value
	No.	Mean ± SD	No.	Mean ± SD	
WBC (10^3/μL)					
Baseline	48	6.05 ± 1.51	48	6.28 ± 1.58	0.4704†
Week 8	43	6.08 ± 1.13	44	6.49 ± 1.62	
Change from baseline	43	0.08 ± 1.48	44	0.22 ± 1.11	0.5637†
RBC (10^6/μL)					
Baseline	48	4.96 ± 0.46	48	4.88 ± 0.40	0.3460*
Week 8	43	4.84 ± 0.49	44	4.81 ± 0.40	
Change from baseline	43	−0.12 ± 0.24	44	−0.06 ± 0.21	0.1729†
Hb (g/dL)					
Baseline	48	15.18 ± 1.35	48	15.06 ± 1.15	0.8173†
Week 8	43	14.77 ± 1.45	44	14.86 ± 1.13	
Change from baseline	43	−0.41 ± 0.70	44	−0.15 ± 0.61	0.0687*
Hct (%)					
Baseline	48	44.86 ± 3.89	48	44.26 ± 3.13	0.4180†
Week 8	43	43.62 ± 4.24	44	43.69 ± 3.34	
Change from baseline	43	−1.33 ± 2.11	44	−0.47 ± 1.85	0.0462*
MCV (FL)					
Baseline	48	90.55 ± 4.03	48	90.91 ± 4.58	0.6799*
Week 8	43	90.34 ± 4.28	44	90.93 ± 4.20	
Change from baseline	43	−0.52 ± 1.99	44	0.02 ± 1.98	0.3287†
Platelet (10^3/μL)					
Baseline	48	254.50 ± 54.01	48	266.90 ± 56.01	0.2725*
Week 8	43	238.35 ± 46.65	44	268.73 ± 57.46	
Change from baseline	43	−16.88 ± 29.75	44	1.45 ± 27.54	0.0037*
Segmented neutrophil (%)					
Baseline	48	53.39 ± 8.73	48	52.16 ± 8.30	0.4800*
Week 8	43	53.09 ± 8.74	44	52.31 ± 8.42	
Change from baseline	43	−0.37 ± 7.26	44	0.87 ± 6.45	0.4018*
Lymphocyte (%)					
Baseline	48	35.78 ± 7.89	48	37.94 ± 7.90	0.1828*
Week 8	43	36.38 ± 8.48	44	37.67 ± 7.60	
Change from baseline	43	0.71 ± 7.34	44	−0.85 ± 5.62	0.2683*
Monocyte (%)					
Baseline	48	6.96 ± 1.44	48	6.61 ± 1.60	0.2236†
Week 8	43	6.97 ± 1.91	44	6.44 ± 1.52	
Change from baseline	43	0.04 ± 1.36	44	−0.23 ± 1.12	0.3250*
Eosinophil (%)					
Baseline	48	3.23 ± 2.80	48	2.68 ± 1.66	0.4635†
Week 8	43	3.36 ± 3.64	44	2.90 ± 2.09	
Change from baseline	43	0.04 ± 1.48	44	0.14 ± 1.53	0.9425†
Basophil (%)					
Baseline	48	0.68 ± 0.29	48	0.63 ± 0.22	0.3472*
Week 8	43	0.71 ± 0.39	44	0.69 ± 0.29	
Change from baseline	43	0.04 ± 0.29	44	0.05 ± 0.24	0.5714†

WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume
Compared between groups: *p value by 2 sample t-test; †p value by Wilcoxon rank sum test.

decreased by 1.33% ± 2.11% (p = 0.0002), and that of the control group decreased by 0.47% ± 1.85% (p = 0.1011). Although differences were borderline significant (p = 0.0462) between groups, the change within groups before and after the study were subtle and within normal range. Platelet count was decreased by 8.88 ± 29.75 ×10^3/μL in the test group (p = 0.0006), and increased by 1.45 ± 27.54 ×10^3/μL in the control group (p = 0.7278), showing statistically significant difference between 2 groups (p = 0.0037). Again, the difference before and after the tests of both groups were within normal range. All the other hematologic parameters did not show significant differences between the 2 groups. The hemochemical tests included

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Table 3. Change of hemochemical parameters

Hemochemical parameters	SPB-201		Placebo		p value
	No.	Mean ± SD	No.	Mean ± SD	
Protein (g/dL)					
Baseline	48	7.50 ± 0.43	48	7.50 ± 0.42	1.0000*
Week 8	43	7.36 ± 0.30	44	7.37 ± 0.39	
Change from baseline	43	−0.15 ± 0.33	44	−0.13 ± 0.27	0.7373*
Albumin (g/dL)					
Baseline	48	4.53 ± 0.25	48	4.53 ± 0.29	0.9057†
Week 8	43	4.42 ± 0.23	44	4.44 ± 0.26	
Change from baseline	43	−0.12 ± 0.20	44	−0.08 ± 0.19	0.3283†
Total bilirubin (mg/dL)					
Baseline	48	0.91 ± 0.40	48	0.84 ± 0.39	0.2588†
Week 8	43	0.83 ± 0.40	44	0.80 ± 0.33	
Change from baseline	43	−0.09 ± 0.23	44	−0.05 ± 0.29	0.4853*
ALP (U/L)					
Baseline	48	166.58 ± 93.94	48	148.15 ± 75.77	0.3953†
Week 8	43	167.79 ± 96.37	44	145.80 ± 73.49	
Change from baseline	43	0.02 ± 20.07	44	−0.70 ± 19.09	0.8953†
Glucose (mg/dL)					
Baseline	48	100.94 ± 13.74	48	105.58 ± 21.27	0.4524†
Week 8	43	102.91 ± 15.07	44	105.39 ± 15.70	
Change from baseline	43	3.44 ± 14.22	44	1.23 ± 11.80	0.5102†
BUN (mg/dL)					
Baseline	48	14.59 ± 3.81	48	13.88 ± 3.90	0.5048†
Week 8	43	14.71 ± 3.75	44	13.95 ± 4.48	
Change from baseline	43	0.06 ± 3.76	44	0.10 ± 3.82	0.9524*
Cr (mg/dL)					
Baseline	48	0.86 ± 0.18	48	0.84 ± 0.17	0.5481*
Week 8	43	0.83 ± 0.16	44	0.82 ± 0.17	
Change from baseline	43	−0.02 ± 0.08	44	−0.01 ± 0.08	0.3678†
UA (mg/dL)					
Baseline	48	6.10 ± 1.75	48	5.98 ± 1.42	0.8575†
Week 8	43	5.96 ± 1.64	44	6.05 ± 1.36	
Change from baseline	43	−0.14 ± 0.80	44	0.04 ± 0.73	0.1996†
Ca (mg/dL)					
Baseline	48	9.39 ± 0.78	48	9.35 ± 0.39	0.3890†
Week 8	43	9.29 ± 0.29	44	9.20 ± 0.33	
Change from baseline	43	−0.10 ± 0.81	44	−0.15 ± 0.31	0.5636†
Na (mmol/L)					
Baseline	48	139.52 ± 2.37	48	139.48 ± 1.77	0.9302*
Week 8	43	139.42 ± 1.88	44	139.32 ± 1.95	
Change from baseline	43	−0.23 ± 2.72	44	−0.31 ± 1.86	0.7693†
K (mmol/L)					
Baseline	48	4.36 ± 0.37	48	4.38 ± 0.33	0.8166*
Week 8	43	4.24 ± 0.34	44	4.33 ± 0.34	
Change from baseline	43	−0.14 ± 0.38	44	−0.06 ± 0.34	0.2844*
Cl (mmol/L)					
Baseline	48	103.26 ± 2.79	48	103.56 ± 2.33	0.5685*
Week 8	43	103.63 ± 2.47	44	103.77 ± 2.11	
Change from baseline	43	0.47 ± 2.66	44	0.02 ± 1.95	0.3752*

ALP, alkaline phosphate; BUN, blood urea nitrogen; Cr, creatinine; UA, uric acid; Ca, calcium; Na, sodium; K, potassium; Cl, chloride.
Compared between groups: *p value by 2 sample t-test; †p value by Wilcoxon rank sum test.

total protein, albumin, bilirubin, alkaline phosphatase (ALP), glucose, blood urea nitrogen (BUN), creatinine (Cr), uric acid (UA), calcium (Ca), sodium (Na), potassium (K), and chloride (Cl) levels. In all of the categories, no statistically significant differences were observed in the study.

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DISCUSSION

It has been reported that *A. annua* L., one of the most commonly distributed wormwood in Korea, has excellent pharmacological efficacy against a wide range of diseases. In the previous studies on HFD-induced fatty liver mouse model, SPB-201 was shown to maintain the normal liver weight of fatty liver mice and inhibited lipid accumulation in the liver. In addition, SPB-201 improved liver function indicators, blood AST, ALT and TG levels by more than 40% and reduced TC to near normal values [25-27]. *A. annua* extract significantly increased the p-AMPK and p-ACC, resulting in suppression of adipogenesis and prevention of lipid accumulation in the liver of HFD-fed mice [25]. The ability of *A. annua* extract in reducing AST and ALT levels and in protecting the hepatocyte from fatal liver damage was further confirmed in the LPS/D-GalN-induced liver failure mouse model. *A. annua* extract has shown to contain strong anti-inflammatory and antioxidant activities, resulting in effective treatment for fatty liver disease and protection from the liver failure [28].

Excess accumulation of fat in the liver accompanying chronic inflammation renders the high risk of progression into chronic liver diseases such as cirrhosis and liver cancer [4,5]. Considering the anti-inflammatory and anti-oxidative actions of *A. annua* from pre-clinical studies, SPB-201 was expected to improve liver function in this clinical study.

We therefore aimed to verify the effectiveness and safety of SPB-201 for 8 weeks in improving liver function in subjects with liver function impairment. The efficacy evaluation variables for liver function were the extent of changes in the levels of the typical liver enzymes AST and ALT, which normally help with protein metabolism and energy generation [29]. AST and ALT are secreted into the blood in cases of liver damage and/or liver diseases.

After 4 weeks of SPB-201 intake, we found a decrease of 271% in AST levels, and a 334% reduction in ALT levels as compared to the control. After 8 weeks of SPB-201 intake, the test group showed an AST reduction of 199% and a significant ALT decrease of 216% as compared to the control group. This demonstrated that SPB-201 intake has the effect of improving the function of AST and ALT. Unlike ALT, which is mainly present only in the liver, AST is widely distributed in the muscles, heart, internal organs, and brain as well. Therefore, ALT can be used as a specific indicator of liver function. In this clinical study, the ALT level of the test group was significantly reduced after 4 weeks as compared to the control group, and decreased progressively until the end of the study, indicating a distinct effect of SPB-201 on improving liver function and maintaining liver health.

The fatigue scale can be used as a measure of liver dysfunction, and the total score for global fatigue severity, situation-specific fatigue, and daily dysfunctional fatigue is evaluated as MFS [30-33]. After 4 weeks and 8 weeks of substance intake, the fatigue score of the test group decreased while that of the control group increased. It can therefore be judged that overall fatigue may be resolved through the improvement of liver function by SPB-201.

In this study, we confirmed the safety of the test substance SPB-201. No significant adverse events were observed in tall subjects during the study and no significant differences in adverse events were observed between groups even though there were 9 adverse events from the 7 test subjects and 14 from the 11 control subjects. In addition, no clinically meaningful side effects related to SPB-201 were observed. Hematological examination showed significantly different changes in Hct between the two groups after 8 weeks of intake

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(p = 0.0462). The normal levels of red blood cell volume (Hct) are 39%–52% for men and 36%–48% for women, and the changes in both groups were minor and within the normal range from 44.86% to 43.62% and 44.26% to 43.69%, respectively. Platelet count decreased by 8.88 ± 29.75 $\times 10^3/\mu L$ in the test group but increased by 1.45 ± 27.54 $\times 10^3/\mu L$ in the control group, showing statistical differences between them (p = 0.0037). The normal platelet count range between 150–450 $\times 10^3/\mu L$ and the platelet levels of both groups before and after the study were within the normal ranges. Besides these, there were no signs of abnormality in the remaining clinical pathology parameters and no differences between the groups. Thus, we confirmed that oral SPB-201 intake for 8 weeks is clinically effective and safe.

CONCLUSION

Taken together, our clinical study confirmed the excellent efficacy and safety of SPB-201 on liver function improvement, showing that SPB-201 can be developed into a functional food to restore damaged liver function such as in non-alcoholic fatty livers, or to treat liver diseases.

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SUPPLEMENTARY MATERIALS

Supplementary Figure 1

Reduction rate of AST.

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Supplementary Figure 2

Reduction rate of ALT.

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