Effect of a Combination of Hot Water Extract of *Curcuma longa* and Curcumin on Serum Liver Enzymes and Emotional States in Healthy Participants with Moderately High Body Mass Index

—A Randomized, Double-blind, Placebo-controlled Clinical Trial—

Kengo Kawasaki[1)]*　Koutarou Muroyama[1)]
Sumio Kondo[2)]　Shinji Murosaki[1)]

ABSTRACT

Objectives We investigated the effect of a combination of hot water extract of *Curcuma longa* and curcumin on liver function and emotional states in healthy participants with moderately high body mass index（BMI）.

Methods A randomized, double-blind, placebo-controlled clinical trial was designed and conducted to evaluate the effects of a 12-week treatment of the combination on serum liver enzyme levels and scores on the Profile of Mood States（POMS）.

Results Throughout the intervention period, aspartate aminotransferase（AST）, alanine aminotransferase（ALT）, and γ-glutamyltransferase（γ-GTP）levels did not differ between groups when all participants were included in the analysis. After excluding participants whose BMI changed substantially during the study period, decreases in AST levels were significantly greater and those in ALT levels tended to be greater in the combination group than in the placebo group for participants whose serum liver enzyme levels were in the normal range（i. e., AST or ALT＜51 U/L）. Compared with the placebo group, the combination group showed a significantly greater improvement in all POMS subscale scores, except for Vigor scores.

Conclusions The combination may be useful for maintaining liver health in people with borderline hepatitis and improving negative emotional states.

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KEY WORDS *Curcuma longa*, Curcumin, ALT, Liver, Emotional states

INTRODUCTION

Non-alcoholic fatty liver disease（NAFLD）is a major cause of chronic liver disease.[1)] NAFLD presents as a broad spectrum of disorders, ranging from simple fatty liver to more severe forms of liver injury, including hepatitis and fibrosis.[2,3)] NAFLD can lead to hepatic cirrhosis and hepatocellular carcinoma.[4)]

Mild to moderate elevation of serum aminotransferases, such as aspartate aminotransferase（AST）or alanine

*Corresponding author
[1)]Research & Development Institute, House Wellness Foods Corporation　[2)]Fukushima Healthcare Center, Kenshokai Medical Corporation

Table 1　Composition of test drinks（per 100 mL）

	Placebo	Combination
Energy（kcal）	21.9	23.1
Protein（g）	0.02	0.06
Fat（g）	0.06	0.01
Carbohydrate（g）	5.5	5.9
Bisacurone（μg）	0	400
Curcumin（mg）	0	30

aminotransferase（ALT）, is the most common laboratory abnormality found in NAFLD patients.[5,6] Liver biopsy is needed for accurate diagnosis of NAFLD after excluding other factors such as the hepatitis virus or alcohol.[3,5]

Reactive oxygen species and inflammatory cytokines such as tumor necrosis factor-α（TNF-α）are thought to be involved in the development of NAFLD and non-alcoholic steatohepatitis（NASH）. Increases in ROS associated with fatty liver cause oxidative stress and cell death. Oxidative stress also triggers production of inflammatory cytokines, which leads to inflammation and a fibrogenic response.[7,8]

Many clinical studies have tested the suitability of antioxidants or anti-inflammatory agents for the treatment of NAFLD/NASH.[9] One meta-analysis showed that pentoxifylline, which decreases the production of proinflammatory cytokines（including TNF-α）, can reduce aminotransferase activities and improve the histological parameters in NAFLD patients.[10] A meta-analysis of randomized controlled trials concluded that antioxidant vitamin E significantly improves liver function and histologic changes in patients with NAFLD/NASH.[11]

Curcuma longa（*C. longa*）is a rhizomatous herbaceous perennial plant in the ginger family. Curcuma is cultured in tropical or subtropical regions worldwide. The deep yellow-orange powder known as turmeric is produced by boiling and drying the rhizomes of *C. longa* and is commonly used as a spice and a drug, especially in Asia.[12]

Water extract of *C. longa* has an anti-inflammatory effect.[13,14] The physiological effects of water extract of *C. longa*, such as cardiovascular protection,[15] anti-stress effects,[16] and neuroprotection,[17] are attributed to its suppressive effect on oxidative stress. Water extract of *C. longa* also has a protective effect against carbon tetrachloride-induced liver toxicity.[18]

Curcumin is a principal compound of *C. longa*. There are numerous reports of curcumin's antioxidant and anti-inflammatory effects.[19] Animal models have demonstrated that curcumin has high therapeutic ability for hepatic disorders including alcoholic and nonalcoholic fatty liver diseases.[20] Numerous clinical studies have been conducted about curcumin.[21] Curcumin at a dose of 250 mg/d combined with 1 g/d of *Tinospora* extract was reported to have preventive effect against hepatotoxicity induced by anti-tuberculosis treatment.[22] However, the dose of curcumin used in the clinical trial exceeds the acceptable daily intake（ADI, 0-3 mg/d/kg）determined by the Joint FAO/WHO Expert Committee on Food Additives at the 61st Meeting, 2003. Therefore, it is not recommended that healthy people consume daily curcumin at doses higher than 3 mg/d/kg for prevention and treatment of liver disease. In this study, the dose of curcumin has been set at 30 mg/d which is substantially below the ADI. Because effects of the low dose of curcumin on liver disease was ambiguous, we combined hot water extract of *C. longa* with the low dose of curcumin in order to clarify the effects.

In this study, we aimed to investigate the effects of a combination of hot water extract of *C. longa* and curcumin on liver function in healthy participants with moderately high body mass index（BMI）. A randomized, double-blind, placebo-controlled clinical trial was designed and conducted to evaluate the effects of a 12-week treatment of the combination on serum liver enzymes. Additionally, we investigated the effects of the combination on emotional states using the Profile of Mood States（POMS）.

MATERIALS AND METHODS

1　Test drinks

Table 1 shows the composition of test drinks. Placebo and combination drinks were made mainly from high-fructose corn syrup, citric acid, ascorbic acid, and flavors. The combination drink contained DR Turmeric Extract PHT, a hot water extract of *C. longa*, which was provided by Takasago International Corporation（Tokyo, Japan）and contained bisacurone, a potential active ingredient. The combination drink also contained Turmeric color HH-1187 which was provided by Inabata Koryo Corporation（Osaka, Japan）and mainly consisted of curcumin. The contents of bisacurone and curcumin in test drinks were confirmed by high-performance liquid chromatography.

2　Participants

Healthy participants were recruited in December 2014 and January 2015 and assessed for eligibility for participation. Inclusion criteria were as follows: 1. age from 20 to 64 years; 2. BMI from 24 to 30; 3. participants with

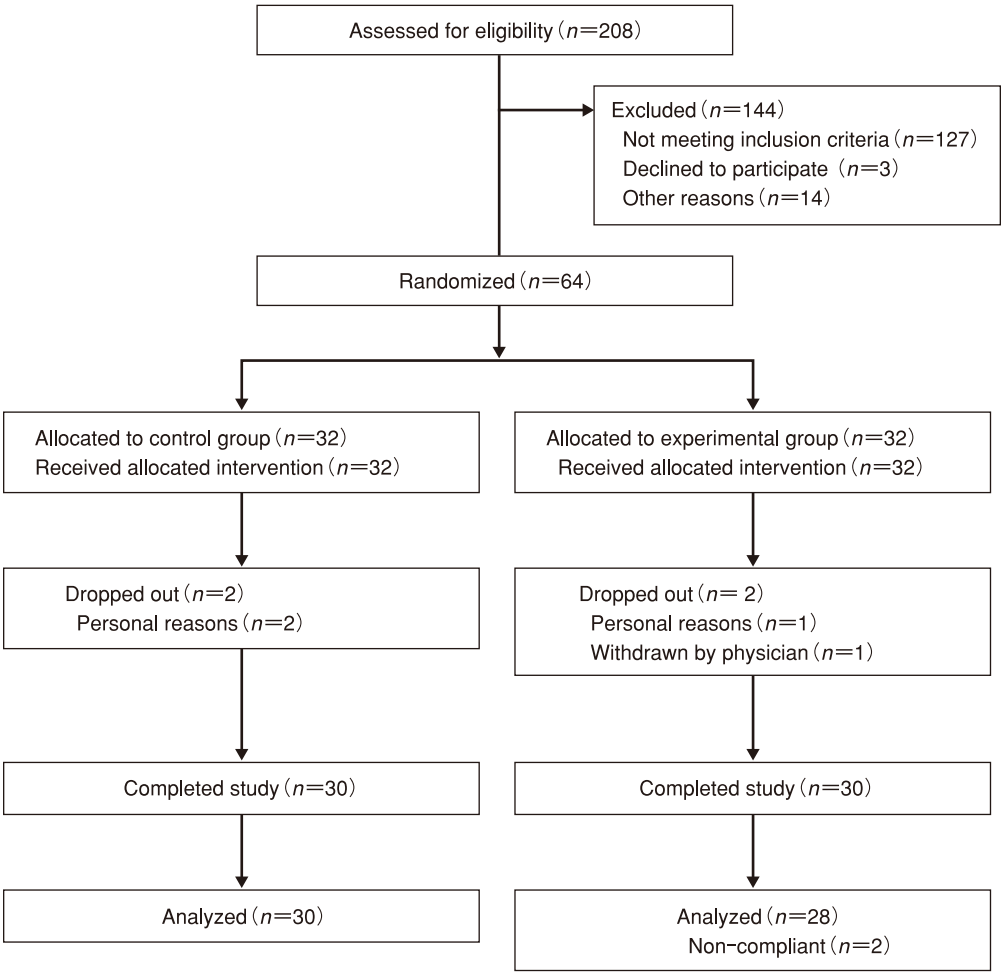

Fig. 1　Flow chart of study participants

higher ALT levels within the normal range（＜51 U/L）. Exclusion criteria were as follows: 1. habitual use of medicines or healthcare foods that could influence liver function; 2. habitual use of medicines or healthcare foods containing *C. longa*; 3. unsuitable lifestyle; 4. allergy against *C. longa*; 5. current history of illness requiring consecutive treatment with a medicine or a previous history of serious disease; 6. marked abnormality in screening laboratory test; 7. participation in another clinical trial; 8. pregnant or lactating; 9. positive for hepatitis C（HCV）antibody or hepatitis B surface antigen; 10. inappropriate participants judged by the investigator. Among 208 healthy participants recruited, 64 participants（48 men and 16 women; mean age 44.4 y）were eligible and randomly assigned（**Fig. 1**）. Eating habits were not included in the criteria, but participants were instructed to continue with their usual diet during the study. Approval of the protocol was obtained from Kenshokai Ethical Review Board（Osaka, Japan）on January 9, 2015, and the study was conducted in accordance with the Declaration of Helsinki of 1975, as revised 2008. The procedures were fully explained to the participants, who provided written informed consent before the beginning of the study.

3　Experimental design

Sixty-four healthy participants were enrolled in a randomized, double-blind, placebo-controlled, parallel study. The participants were randomly assigned to two groups using a sequential series of numbered sealed envelopes that each contained one food ingredient assigned in a computer-randomized manner. After the assignment and a 4-week pre-treatment observation period, the participants consumed either a 100 mL drink containing hot water extract of *C. longa* and curcumin or a matching control drink once daily for 12 weeks. Throughout the study, the participants rated themselves once daily in a subject diary to assess their general health, days of medication, and compliance with the dietary regimens. Anthropometric measurements, bio-

chemical examinations of blood, hematological assessments, and urine tests were performed at 0, 4, 8, and 12 weeks during the intake period and 4 weeks after the end of the intake period. Blood and urine tests were completed by a clinical laboratory testing company, LSI Medience（Tokyo, Japan）. The study was conducted at Fukushima Healthcare Center, Kenshokai Medical Corporation and Gou Clinic（Osaka, Japan）from January 2015 to July 2015.

4　Stratified analysis

Participants were stratified according to their liver status. Participants whose serum AST＜51, ALT＜51, and γ-glutamyltransferase（γ-GTP）＜101 were considered in the normal range and were further stratified into two groups. Participants whose serum AST＞30, ALT＞30, or γ-GTP＞50 were considered in the borderline range. Participants whose serum AST＜31, ALT＜31, and γ-GTP＜51 were considered in the sound range. The stratification was determined by reference to the 'Program for Standard Health Check-ups and Standard Health Guidance' which was published in April 2013 by Ministry of Health, Labour and Welfare, Japan.

5　Profile of Mood States

We analyzed participants'emotional states using the POMS, which was developed to assess transient distinct mood states.[23] Yokoyama translated the original form into Japanese and developed a brief version of the POMS, which comprises 30 items and thus reduces patient burden. The reliability and validity of the POMS-Brief for Japanese participants has been demonstrated.[24] Each item is rated on a scale of 0 to 4, ranging from "not at all" to "extremely". The self-rating results are used to calculate scores on six emotional states: Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor, Fatigue, and Confusion. A seventh score of total mood disturbance is calculated by subtracting the score of the one positively scored subscale（Vigor）from the sum of the other five subscales.

6　Statistical analysis

We compared the baseline values between groups using the unpaired *t*-test. Changes in AST, ALT, and γ-GTP levels, and in POMS scores from baseline, were analyzed using two-way ANOVA, followed by comparison between the two groups at each time point using the unpaired *t*-test. Although the data obtained over time are suitably analyzed by repeated measures ANOVA which incorporates the correlation structure, they were analyzed by two-way ANOVA because we have assumed that the data can be regarded as independent over time. Pearson's correlation coefficient was used to analyze the correlation between BMI changes from baseline and ALT changes from baseline. All analyses were performed using Statcel2 software（OMS Publishing, Tokorozawa, Japan）. A probability value of $P<0.05$ was considered to indicate statistical significance.

RESULTS

1　Baseline characteristics

Before completing the study, two participants in the placebo group dropped out, one because of relocation, and another because of treatment for psychiatric illness. Two participants in the combination group dropped out, one because of relocation, and another because of elevation of serum liver enzyme levels. In addition, two participants in the combination group who completed the study were excluded from the analysis, one because of pollinosis and its drug treatment, and another because of many subjective symptoms（including pyrexia, headache, and malaise）and its drug treatment. Fifty-eight participants were included in the efficacy evaluation. The baseline characteristics are shown in **Table 2**. No parameters were significantly different between the two groups, except POMS fatigue scores.

2　ALT, AST, and γ-GTP levels

Changes in AST, ALT, and γ-GTP levels from baseline to each time point are shown in **Table 3**. Between the placebo and the combination groups, there were no significant differences in the changes of AST, ALT, and γ-GTP levels from baseline.

3　Assessment of the correlation between BMI and ALT

Within the placebo group, weight and BMI tended to decrease at week 4 and significantly decreased at week 8 and week 12（data not shown）. Decreases in BMI in the placebo group tended to be greater at week 8 and were significantly greater at week 12 than those in the combination group（**Table 4**）. The changes in BMI from baseline at week 12 were significantly correlated with changes in ALT from baseline at week 12（Pearson correlation: $n=58$, $r=0.324$, $P=0.006$）. As the decrease in BMI contributed to the decrease in ALT, and as BMI was significantly decreased in the placebo group, we evaluated the efficacy of the combination on serum liver enzymes after excluding a quarter of the participants whose BMI changed substantially during the study period in order to remove the influence of BMI changes.

Table 2　Baseline characteristics of enrolled participants

	Placebo (*n* or mean±SD)	Combination (*n* or mean±SD)	*P*-value
n	30	28	
Sex, male: female	22：8	21：7	
Age, y	45.3±8.9	44.2±7.9	0.61
Weight（kg）	76.4±8.6	74.1±8.8	0.32
BMI（kg/m^2）	27.2±1.9	27.0±1.5	0.61
AST（U/L）	26.8±8.4	25.8±6.5	0.61
ALT（U/L）	31.7±11.4	32.9±11.6	0.71
γ-GTP（U/L）	43.4±27.6	42.9±30.3	0.95
POMS			
Tension-Anxiety	41.3±9.2	42.9±10.6	0.53
Depression-Dejection	44.3±5.7	46.4±8.8	0.30
Anger-Hostility	43.1±8.2	47.0±10.4	0.12
Vigor	43.2±10.5	45.3±9.4	0.41
Fatigue	42.2±6.8	47.0±9.6	0.03
Confusion	47.2±8.3	48.9±8.5	0.46
Total Mood Disturbance	175±33	189±43	0.24

Note: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase; POMS, Profile of Mood States.

Table 3　Changes in serum liver enzyme levels from baseline

	Week 4 (U/L mean±SD)	Week 8 (U/L mean±SD)	Week 12 (U/L mean±SD)	2-way ANOVA		
				Intervention	Time	Interaction
					P-value	
AST						
Placebo	−2.47±8.13	−1.97±6.38	−1.67±7.61	0.20	0.42	0.70
Combination	−1.64±5.55	−1.32±5.42	0.96±8.52			
ALT						
Placebo	−1.80±10.6	0.13±10.8	0.27±10.1	0.21	0.17	0.66
Combination	−0.93±7.19	1.14±7.72	4.14±12.2			
γ-GTP						
Placebo	−3.37±13.0	−3.87±14.8	−3.20±12.6	0.51	0.57	0.59
Combination	−4.86±15.2	−2.04±8.75	0.57±17.1			

Note: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase. $n=30$ for the placebo group and $n=28$ for the combination group.

Table 4　Changes in BMI from baseline

	Week 4（mean±SD）	Week 8（mean±SD）	Week 12（mean±SD）
BMI（kg/m^2）			
Placebo	−0.13±0.37	−0.32±0.54	−0.54±0.62
Combination	−0.02±0.36	−0.09±0.45$^{\$}$	−0.14±0.58*

Note: $^{\$}0.05<P<0.1$, $^{*}P<0.05$, compared with placebo group. $n=30$ for the placebo group and $n=28$ for the combination group.

Table 5　Changes in serum liver enzyme levels from baseline for participants whose BMI was relatively maintained

	Week 4 (U/L mean±SD)	Week 8 (U/L mean±SD)	Week 12 (U/L mean±SD)	2-way ANOVA		
				Intervention	Time	Interaction
				P-value		
AST						
Placebo	−1.81±7.92	−0.48±6.20	−0.86±8.13	0.33	0.86	0.85
Combination	−2.27±5.72	−2.50±5.26	−1.73±5.64			
ALT						
Placebo	−0.14±8.08	2.86±8.89	1.24±11.3	0.35	0.59	0.72
Combination	−0.91±7.00	−0.32±7.13	0.86±9.47			
γ-GTP						
Placebo	−2.48±10.9	−1.86±13.0	−2.10±12.4	0.74	0.85	0.92
Combination	−3.05±6.99	−1.59±7.29	−3.64±10.4			

Note: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase. $n=21$ for the placebo group and $n=22$ for the combination group.

Table 6　Stratified analysis in participants whose BMI was relatively maintained

	Week 4 (U/L mean±SD)	Week 8 (U/L mean±SD)	Week 12 (U/L mean±SD)	2-way ANOVA		
				Intervention	Time	Interaction
				P-value		
Participants whose serum liver enzyme levels were in the normal range[1]						
AST						
Placebo	−0.05±5.04	0.58±4.73	0.63±5.48	0.015	0.77	0.76
Combination	−1.89±4.86	−2.79±5.15*	−1.26±5.31			
ALT						
Placebo	1.53±6.14	3.79±8.78	2.68±10.5	0.054	0.87	0.55
Combination	−0.11±6.84	−1.58±6.78*	0.68±9.38			
γ-GTP						
Placebo	−0.37±7.70	−0.11±9.57	0.05±10.6	0.36	0.99	0.99
Combination	−1.74±6.26	−1.37±7.49	−1.74±8.78			
Participants whose serum liver enzyme levels were in the borderline range[2]						
AST						
Placebo	0.11±7.24	1.22±5.70	0.89±4.81	0.013	0.95	0.79
Combination	−2.55±5.07	−3.82±5.13[$]	−2.55±5.30			
ALT						
Placebo	2.11±8.45	4.67±11.5	4.67±9.58	0.018	0.94	0.64
Combination	−0.64±7.09	−3.36±7.12[$]	−1.64±9.94			
γ-GTP						
Placebo	−0.22±10.6	−0.78±13.9	0.11±14.8	0.53	0.99	0.95
Combination	−2.73±6.89	−1.27±9.24	−2.36±11.4			
Participants whose serum liver enzyme levels were in the sound range[3]						
AST						
Placebo	−0.20±2.04	0.00±3.89	0.40±6.28	0.59	0.75	0.89
Combination	−1.00±4.75	−1.38±5.15	0.50±5.13			
ALT						
Placebo	1.00±3.37	3.00±6.00	0.90±11.4	0.94	0.83	0.58
Combination	0.63±6.89	0.88±5.84	3.88±8.06			
γ-GTP						
Placebo	−0.50±4.22	0.50±3.37	0.00±5.48	0.46	1.0	0.78
Combination	−0.38±5.42	−1.50±4.72	−0.88±3.56			

Note: ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase. *$0.01<P<0.05$, compared with placebo group. [1]$n=19$ for the placebo group and $n=19$ for the combination group. [2]$n=10$ for the placebo group and $n=8$ for the combination group. [3]$n=9$ for the placebo group and $n=11$ for the combination group.

Table 7　Changes in Profile of Mood States（POMS）scores from baseline

	Week 4 (mean±SD)	Week 8 (mean±SD)	Week 12 (mean±SD)	2-way ANOVA		
				Intervention	Time	Interaction
Tension-Anxiety						
Placebo	3.40±7.19	2.00±5.62	3.40±11.5	0.0012	0.82	0.82
Combination	−0.54±6.70*	−0.82±6.53	−1.14±6.11			
Depression-Dejection						
Placebo	2.40±8.79	2.03±7.15	4.37±12.3	0.0052	0.85	0.40
Combination	0.39±7.03	−0.57±5.03	−1.43±5.49*			
Anger-Hostility						
Placebo	2.03±5.38	1.83±7.20	3.47±11.0	0.0024	0.73	0.82
Combination	−1.39±6.73*	−0.75±6.56	−0.82±5.57			
Vigor						
Placebo	0.10±9.98	0.50±9.18	−0.57±8.08	0.52	0.44	0.62
Combination	−2.32±4.73	1.00±7.73	−1.04±7.99			
Fatigue						
Placebo	3.10±6.00	2.97±8.43	4.63±8.59	0.00087	0.92	0.56
Combination	0.36±6.34	−0.18±6.32	−0.86±7.99*			
Confusion						
Placebo	0.63±7.89	0.80±7.67	3.63±11.8	0.0053	0.14	0.64
Combination	−1.11±6.57	−3.25±3.65*	−0.50±5.84			
Total mood disturbance						
Placebo	11.5±26.8	9.13±31.1	20.1±49.5	0.00045	0.47	0.56
Combination	0.04±24.0	−6.57±21.4*	−3.71±24.6*			

Note: *$0.01 < P < 0.05$, compared with placebo group. $n = 30$ for the placebo group and $n = 28$ for the combination group.

4　Stratified analysis

Changes from baseline in serum AST, ALT, and γ-GTP for participants whose BMI was relatively maintained are shown in **Table 5**. There were no differences between the placebo and the combination groups in the changes to AST, ALT, and γ-GTP levels. However, for participants whose serum liver enzyme levels were in the normal range, the decreases in AST and ALT levels from base line to week 8 were significantly greater in combination group than in placebo group. In addition, the decreases in AST levels were significantly greater in the combination group than in the placebo group（$P = 0.015$）and those in ALT levels tended to be greater（$P = 0.054$）over the study period but there were no interaction between the intervention and the time（**Table 6**）. Moreover, for participants whose serum liver enzyme levels were in the borderline range, the decreases in AST and ALT levels from baseline to week 8 were greater in the combination group than in the placebo group. The decreases in AST and ALT levels were significantly greater in the combination group than in the placebo group（$P = 0.013$ and 0.018, respectively）over the study period but there were no interaction between the intervention and the time（**Table 6**）. However, in the participants whose serum liver enzyme levels were in the sound range, the changes in AST, ALT, and γ-GTP levels did not differ between the two groups（**Table 6**）.

5　POMS scores

The changes from baseline at each time point in POMS scores are shown in **Table 7**. The decreases from baseline at week 4 in Tension-Anxiety and Anger-Hostility, at week 8 in Confusion and total mood disturbance, and at week 12 in Depression-Dejection, Fatigue, and total mood disturbance were significantly greater in the combination group than in the placebo group. In addition, decreases in all the scores（except Vigor scores）were significantly greater in the combination group than in the placebo group over the study period but there were no interaction between the intervention and the time.

6　Safety assessment

Thirty-five adverse events（AEs）were recorded during the study. There were eight AEs in the placebo group: headache（2）, diarrhea（2）, common cold symptoms（1）, malaise（1）, epistaxis（1）, and abdominal pain（1）. There were 27 AEs in the combination group: common cold symptoms（8）; diarrhea（2）; fatigue（2）; abdominal pain and diarrhea（1）; seasonal allergic rhinitis（1）; elevation of serum AST, ALT, γ-GTP, and lactate dehy-

drogenase levels（1）; muscular pain（1）; sneezing and runny nose（1）; hangover（1）; headache and pyrexia（1）; pyrexia（1）; indigestion（1）; tonsillitis（1）, nausea, heartburn, headache, backache, malaise, and pharyngeal pain（1）; metrorrhagia（1）; leg pain and edema（1）; insect sting（1）; and headache（1）. All AEs were judged to be mild. Elevation of serum AST, ALT, γ-GTP, and lactate dehydrogenase levels was judged to be probably unrelated to the dietary intervention. Except for these elevated levels, changes revealed in all the physical or clinical examinations ranged within corresponding reference values. All the other AEs were judged by a physician to be unrelated to the dietary intervention.

DISCUSSION

A randomized, double-blind, placebo-controlled clinical trial was designed and conducted to evaluate the effects of a 12-week intake of a combination of hot water extract of *C. longa* and curcumin on serum liver enzymes in healthy participants with moderately high BMI. Throughout the intervention period, AST, ALT, and γ-GTP levels did not differ between groups when all participants were included in the analysis. For participants whose BMI was relatively maintained throughout the study period and whose serum liver enzyme levels were in the normal range, AST levels were significantly decreased and ALT levels tended to decrease in the combination group compared to the placebo group. In addition, AST and ALT levels were significantly decreased in the combination group compared to the placebo group for participants whose serum liver enzyme levels were in the borderline range. Liver enzymes such as ALT are often used as markers of hepatic function and their serum increase has been used as a marker to screen for liver damage.[6,25,26] Therefore, our results suggest that the combination may be useful for prevention and treatment of borderline hepatitis.

In this study, the effect of the combination was shown only in participants whose liver enzyme levels were in the normal or borderline ranges. Even within the normal range, higher ALT levels are correlated with a greater cumulative incidence rate of diabetes.[27] It is possible that the combination has a preventive effect on the progression of liver dysfunction or other pathology in healthy people whose liver functions are within normal levels, whether or not they have risk factors for liver disease（e. g., alcohol consumption, obesity, smoking, or elevated age）. In the participants whose serum liver enzyme levels were in the sound range, the changes in serum liver enzyme levels did not differ between groups, suggesting that the combination was effective for healthy livers with some degree of damage. To clarify the clinical importance of the combination, it is necessary to investigate its effect on people whose serum liver enzyme levels are higher, or on patients in various stages of liver disease caused by different pathologies.

Some antioxidants and anti-inflammatory agents can decrease serum liver enzyme levels and improve liver diseases.[9,28] Because water extract of *C. longa*[13,14,17] and curcumin[19] has antioxidant and anti-inflammatory properties, it is possible that the combination decreased serum AST and ALT levels by suppressing oxidative and inflammatory stress in the liver.

To date, at least 235 compounds, primarily phenolic compounds and terpenoids, have been identified from *C. longa*.[29] Bisacurone is one of these compounds[30] and its anti-inflammatory effects have been reported.[31] Similarly to bisacurone, hot water extract of *C. longa* inhibits vascular cell adhesion molecule VCAM-1 expression induced by TNF-α in endothelial cells through the inhibition of signaling of the protein complex NF-κB, which implies that bisacurone is a potential active ingredient of hot water extract of *C. longa*.[14] We recently affirmed that hot water extract of *C. longa* as well as bisacurone inhibits ethanol-induced liver injury in mice, possibly via inhibition of oxidative stress and inflammation（unpublished data）. Therefore, the fact that the daily intake of hot water extract of *C. longa*, which contained 400 μg bisacurone, was effective in the maintenance of liver function in humans is consistent with the results of animal studies.

The POMS scores indicated that intake of the combination of hot water extract of *C. longa* and curcumin can improve total mood disturbance and the five negative emotional states of tension-anxiety, depression-dejection, anger-hostility, fatigue, and confusion. It has been shown that negative emotional states such as fatigue or depression are related to inflammation in the brain[32] or the whole body.[33] Liver diseases such as primary biliary cirrhosis, HCV,[34] and NAFLD[35] are often accompanied by behavior alterations that include fatigue, mood disorders, cognitive dysfunction, and sleep disturbances. It has also been suggested that in patients with HCV not undergoing interferon-α treatment, liver disease is associated with inflammation in the basal ganglia region.[36] In addition, systemic cytokines and chemokines produced by the liver are thought to play a key role in promoting inflammation within the brain in animal models.[37] In this study, the combination may suppress negative emotional states by inhibiting inflammation in the liver, brain, or the whole body through its anti-inflammatory effects,

even though the participants in this study were healthy, and their inflammation levels were probably much lower than those of patients or animals in the above-mentioned studies.

CONCLUSIONS

In conclusion, we have demonstrated that daily intake of the combination of hat water extract of *C. longa* and curcumin decreased liver enzyme levels in parallel with improvement of POMS score. The present results suggest that intake of the combination may be useful for the maintenance and improvement of liver health and emotional states.

【Competing interests】 The authors declare that they have no competing interests.

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