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

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

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ABSTRACT

Background The aim of this study was to demonstrate the effects of this combination on serum liver enzymes, emotional states, and inflammatory markers to clarify the contribution of systemic inflammation to serum liver enzymes and emotional states.

Methods Eighty healthy participants with moderately high body mass index were assigned to a 12-week treatment with a combination of hot water extract of *Curcuma longa*（400 μg as bisacurone）and curcumin（30 mg）or placebo in a randomized, double-blind, placebo-controlled design.

Results For all participants, serum liver enzyme levels did not significantly differ between the combination and the placebo groups. However, for participants whose serum liver enzyme levels were in the borderline range, decreases in serum liver enzymes（alanine aminotransferase, aspartate aminotransferase, and γ-glutamyltransferase）were significantly greater in the combination group than in the placebo group（P＝0.047, 0.035, and 0.0014, respectively）. Although there were no significant differences in changes in inflammatory marker levels between the placebo and the combination groups, these levels showed a significant decrease in participants whose serum liver enzyme levels had improved. The combination group showed a significantly greater decrease in fatigue scores on the Profile of Mood States（POMS）than the placebo group（P＝0.0007）.

Conclusions This study showed that a combination of hot water extract of *Curcuma longa* and curcumin significantly decreased serum liver enzyme levels of participants with borderline serum liver enzyme levels, possibly through the suppression of systemic inflammation. Additionally, the combination significantly decreased POMS fatigue scores.

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KEY WORDS　*Curcuma longa*, Bisacurone, Liver, Inflammation, Emotional states

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BACKGROUND

Non-alcoholic fatty liver disease（NAFLD）includes a broad spectrum of disorders, ranging from simple fatty liver to more severe forms of liver injury, such as hepatitis and fibrosis.[1,2] Mild to moderate elevation of serum aminotransferases, such as aspartate aminotransferase（AST）or alanine aminotransferase（ALT）, is the most common laboratory abnormality found in NAFLD patients, although liver biopsy is needed for accurate diagnosis.[2–4]

Inflammatory cytokines such as tumor necrosis factor-α（TNF-α）or interleukin-6（IL-6）induce hepatocyte apoptosis and play a key role in hepatic injury in chronic liver diseases.[5] Systemic inflammation is thought to contribute to the progression of chronic liver diseases, for example, serum endotoxin from intestinal microbiota or inflammatory cytokines from adipocyte induce liver inflammation across the organs.[6] NAFLD and non-alcoholic steatohepatitis（NASH）patients have increased levels of serum inflammatory cytokines such as TNF-α and IL-6 and acute phase reactants such as C-reactive protein（CRP）.[7–9] One meta-analysis showed that pentoxifylline, which decreases the production of inflammatory cytokines, can reduce aminotransferase activities and improve histological parameters in NAFLD patients.[10] Anti-TNF-α antibody improves hepatic steatosis in severe alcoholic hepatitis patients.[11]

Curcuma longa（*C. longa*）is a rhizomatous herbaceous perennial plant of the ginger family whose dried rhizomes are widely used as a spice or drug, especially in Asia.[12] In vitro and animal models show that water extract of *C. longa* has an anti-inflammatory effect.[13,14] Animal models have also demonstrated that water extract of *C. longa* has a protective effect against carbon tetrachloride-induced liver toxicity[15] and alcohol-induced liver injury by inhibiting hepatic inflammatory cytokine production.[16] Curcumin, the principal compound of *C. longa*, is usually made from organic solvent extract of *C. longa*. There are numerous reports of curcumin's antioxidant and anti-inflammatory effects.[17] Animal models have also demonstrated that curcumin has high therapeutic efficacy for hepatic disorders, including alcoholic and non-alcoholic fatty liver diseases.[18]

Recently, we have demonstrated that daily intake of a combination of hot water extract of *C. longa* and curcumin can decrease liver enzyme levels in healthy participants[19]; however, the therapeutic mechanism of this effect is unclear. In this study, we aimed to demonstrate the effects of the combination on serum liver enzymes and inflammatory markers to clarify the contribution of systemic inflammation to liver enzyme levels. 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 and inflammatory markers in healthy participants with moderately high body mass index（BMI）.

Additionally, we aimed to demonstrate the effects of the combination on emotional states using the Profile of Mood States（POMS）, assuming that the combination improves emotional states by suppressing systemic[20] or brain[21] inflammation.

Table 1　Composition of test tablets（per three tablets）

	Placebo	Combination
Energy（kcal）	5.3	5.1
Protein（g）	0.03	0.05
Fat（g）	0.01	0.02
Carbohydrate（g）	1.3	1.2
Bisacurone（μg）	0	400
Curcumin（mg）	0	30

METHODS

1　Test tablets

Table 1 shows the composition of test tablets. Placebo and combination tablets were made mainly from maltose and B vitamins. The combination tablet 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 tablet also contained Turmeric Color, which was provided by Inabata Koryo Corporation（Osaka, Japan）and mainly consisted of curcumin. The contents of bisacurone and curcumin in test tablets were confirmed by high-performance liquid chromatography.

2　Participants

Healthy participants were recruited in December 2015 and January 2016 and assessed for eligibility for participation. Inclusion criteria were as follows: 1. age greater than or equal to 20 years; 2. BMI from 24 to 30; 3. AST and ALT within the normal levels（＜51 U/L）. Exclusion criteria were as follows: 1. Positive hepatitis C antibody or hepatitis B surface antigen test results; 2. systolic blood pressure lower than 90 mmHg; 3. possibly pregnant, or lactating; 4. a blood

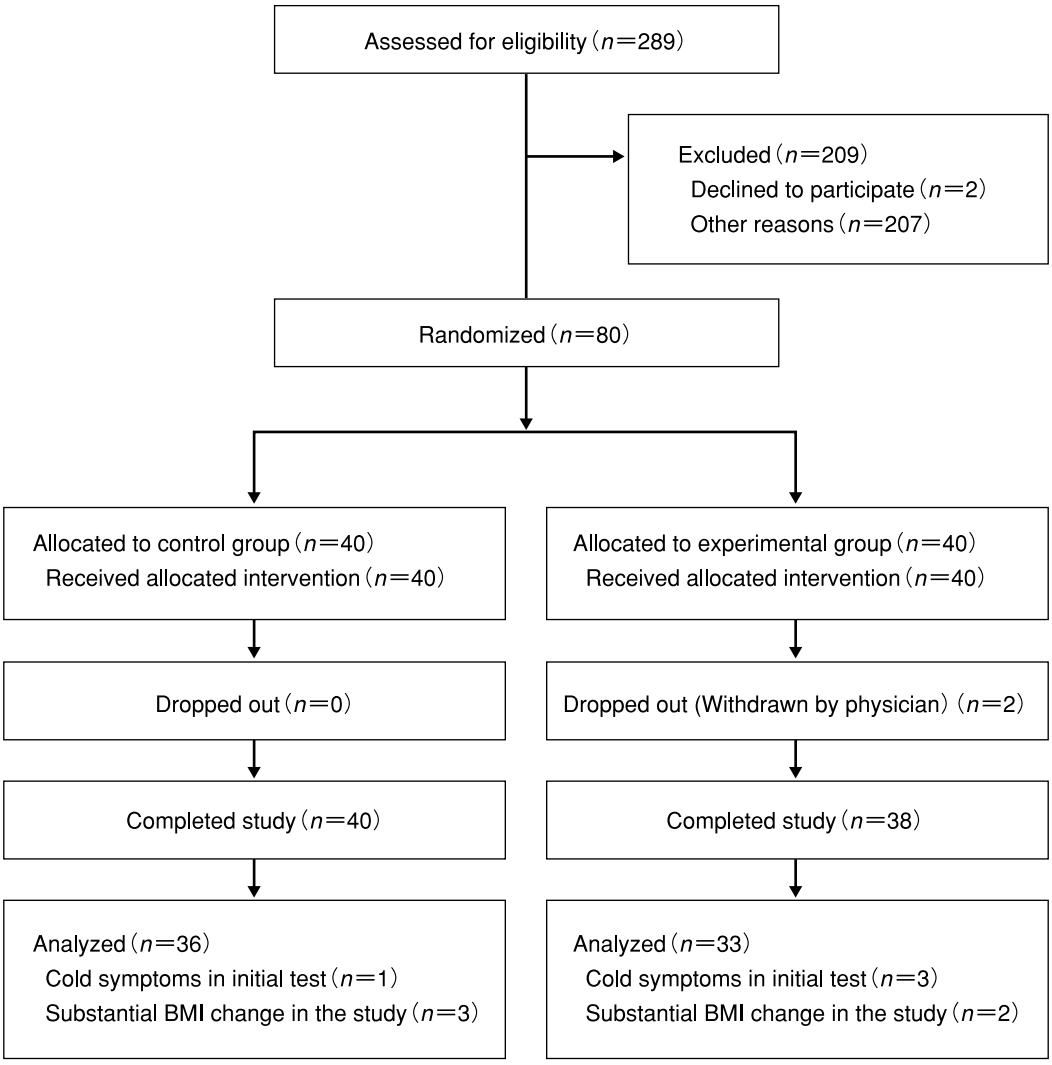

Fig. 1　Flow chart of study participants

donation of 200 mL within the last month prior to this study; 5. for male participants, a blood donation of 400 mL within the last 3 months prior to this study; 6. for female participants, a blood donation of 400 mL within the last 4 months prior to this study; 7. for male participants, a blood donation over 1200 mL（minus the estimated volume of blood sampling during the study）within the last year prior to this study; 8. for female participants, a blood donation over 800 mL（minus the estimated volume of blood sampling during the study）within the last year prior to this study; 9. current participation in another clinical study or had finished another clinical study within the last 4 weeks; 10. heart, liver, or kidney disease; 11. any history of cardiovascular disease; 12. diabetes mellitus; 13. drug allergy or food allergy; 14. excessive alcohol drinking or smoking habit; 15. extremely irregular dietary habits; 16. consecutive intake of pharmaceutical or health care foods that could influence the study results; 17. judged as unsuitable for the study by the investigator. Among 289 healthy participants recruited, 80 participants（40 men and 40 women; mean age 51.7 years）were eligible and randomly assigned（**Fig. 1**）. Approval of the protocol was obtained from Miyawaki Orthopedic Clinic Ethical Review Board（Hokkaido, Japan）on December 8, 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

Eighty 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 three tablets containing hot water extract of *C. longa* and curcumin or matching control tablets 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, biochemical blood analysis, 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, SRL Inc.（Hokkaido, Japan）. The study was conducted at Fukuhara clinic（Hokkaido, Japan）from January 2016 to July 2016.

4　Stratified analysis

Participants were stratified according to their liver status. Participants with serum AST＜51 and ALT＜51 at 0 week were considered in the normal range and were further stratified into two groups. Participants with serum AST＞30 or ALT＞30 at 0 week were considered in the borderline range. Participants with serum AST＜31 and ALT＜31 at 0 week were considered in the sound range. The stratification was determined by reference to the "Program for Standard Health Check-ups and Standard Health Guidance," published in April 2013 by the 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.[22] Yokoyama has 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.[23] 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 unpaired *t*-tests. Changes in AST, ALT, and γ-glutamyltransferase（γ-GTP）levels, and in POMS scores from baseline, were analyzed using two-way analysis of variance（ANOVA）, followed by comparisons between the two groups at each time point using unpaired *t*-tests. Although the longitudinal data could have been analyzed using repeated measures ANOVA（which can incorporate correlations between multiple responses for the same participant）, we used two-way ANOVA because we assumed that the data could be regarded as independent over time. Changes in CRP, serum amyloid protein A（SAA）, IL-6, TNF-α, and prostaglandin E2（PGE2）levels from baseline to week 12 were compared between the two groups using unpaired *t*-tests. All analyses were performed using Statcel2 software（OMS Publishing, Saitama, Japan）. A probability value of $P<0.05$ was considered to indicate statistical significance.

RESULTS

1　Baseline characteristics

As shown in **Fig. 1**, two participants dropped out before completing the study, one because of the possibility of herpes zoster, and another because of ventricular fibrillation. Four participants who completed the study were excluded from the analysis because of cold symptoms with drug treatment and elevation of CRP and SAA at the baseline. In addition, five were excluded because of substantial BMI changes during the study period. Sixty-nine participants were included in the efficacy evaluation. The baseline characteristics are shown in **Table 2**. No parameters were significantly different between the two groups.

2　Serum liver enzyme levels

The changes in AST, ALT, and γ-GTP levels from baseline to each time point are shown in **Table 3**. The decrease in γ-GTP level was greater in the combination group than in the placebo group over the study period（$P=0.051$）, but there was no interaction between the intervention and time. In addition, the decrease in γ-GTP level from baseline to week 12 was greater in the combination group than in the placebo group.

Table 2　Baseline characteristics of participants in efficacy evaluation

	Placebo (n=36)	Combination (n=33)	P-value
Sex, male: female	18：18	17：16	
Age, years	51.3±9.6	51.8±9.8	0.82
BMI（kg/m^2）	26.8±1.6	26.6±1.5	0.61
AST（U/L）	26.0±6.1	24.8±5.2	0.38
ALT（U/L）	31.3±11.2	30.0±11.4	0.63
γ-GTP（U/L）	35.5±17.7	39.1±20.0	0.43
CRP（mg/dL）	0.14±0.09	0.16±0.36	0.29
SAA（μg/mL）	5.0±3.6	7.0±9.4	0.30
IL-6（pg/mL）	1.8±0.9	1.7±1.9	0.49
TNF-α（pg/mL）	1.0±0.2	0.9±0.3	0.33
PGE2（pg/mL）	599±279	589±206	0.40
POMS scores			
Tension-Anxiety	42.4±7.0	42.9±6.3	0.75
Depression-Dejection	45.2±5.6	45.4±5.0	0.86
Anger-Hostility	46.2±6.0	45.5±6.8	0.65
Vigor	45.8±9.3	43.4±7.8	0.26
Fatigue	45.9±6.5	46.2±7.4	0.83
Confusion	46.2±7.7	46.9±7.7	0.71
Total mood disturbance	180±30	184±25	0.61

Mean±SD

SD, standard deviation; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase; CRP, C-reactive protein; SAA, serum amyloid protein A; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α ; PGE2, prostaglandin E2; POMS, Profile of Mood States

Table 3　Changes in serum liver enzyme levels from baseline

	Week 4 (U/L)	Week 8 (U/L)	Week 12 (U/L)	P-value		
				Intervention	Time	Interaction
AST						
Placebo	1.3±4.6	1.8±4.9	2.8±5.7	0.57	0.99	0.27
Combination	2.1±5.6	1.7±5.4	0.8±5.0			
ALT						
Placebo	2.1±10.1	0.8±9.3	2.9±11.8	0.36	0.60	0.64
Combination	2.1±9.1	0.1±8.4	0.0±7.7			
γ-GTP						
Placebo	1.1±8.0	0.6±7.5	0.2±9.0	0.051	0.51	0.70
Combination	−1.5±13.0	−0.9±13.0	−4.3±12.3$^{\$}$			

Mean±SD

SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase

$^{\$}0.05<P<0.1$, compared with placebo group

n=36 for the placebo group and n=33 for the combination group

3　Stratified analysis

For participants whose serum liver enzyme levels were in the normal range, the decrease in γ-GTP level was significantly greater in the combination group than in the placebo group over the study period（P=0.025）, but there was no interaction between the intervention and time（**Table 4**）. In addition, the decrease in γ-GTP level from baseline to week 12 was greater in the combination group than in the placebo group. For participants whose serum liver enzyme levels were in the borderline range, decreases in AST, ALT, and γ-GTP levels were significantly greater in the combination group than in the placebo group over the study period（P=0.047, 0.035, and 0.0014, respectively）, but there

Table 4　Changes in serum liver enzyme levels from baseline: stratified analysis

	Week 4 (U/L)	Week 8 (U/L)	Week 12 (U/L)	*P*-value		
				Intervention	Time	Interaction
Participants whose serum liver enzyme levels were in the normal range[1]						
AST						
Placebo	1.3±4.7	1.9±4.9	2.7±5.8	0.59	0.98	0.36
Combination	2.1±5.7	1.7±5.5	0.9±5.0			
ALT						
Placebo	1.8±8.7	1.0±7.2	2.8±10.0	0.24	0.64	0.57
Combination	1.8±9.1	−0.2±8.3	−0.4±7.6			
γ-GTP						
Placebo	0.8±7.3	0.8±7.0	0.3±8.7	0.025	0.58	0.75
Combination	−1.9±13.1	−1.7±12.4	−4.7±12.4$			
Participants whose serum liver enzyme levels were in the borderline range[2]						
AST						
Placebo	1.5±6.4	3.3±6.1	2.4±8.3	0.047	0.82	0.67
Combination	0.6±5.0	0.0±4.6	−1.0±3.6			
ALT						
Placebo	2.2±12.3	2.5±9.0	2.5±13.4	0.035	0.84	0.79
Combination	−0.5±9.7	−3.2±7.6$	−3.5±8.1			
γ-GTP						
Placebo	0.4±6.0	1.2±4.3	0.1±10.4	0.0014	0.60	0.78
Combination	−5.9±14.5	−6.1±10.9*	−10.1±14.1*			
Participants whose serum liver enzyme levels were in the sound range[3]						
AST						
Placebo	1.1±3.5	1.1±3.9	2.9±3.7	0.15	0.78	0.42
Combination	3.4±6.2	3.2±5.9	2.5±5.6			
ALT						
Placebo	1.6±5.8	0.0±6.0	3.0±7.7	0.32	0.55	0.59
Combination	3.8±8.4	2.4±8.2	2.4±6.2			
γ-GTP						
Placebo	1.0±8.2	0.5±8.4	0.4±7.8	0.69	0.87	0.90
Combination	1.6±10.9	2.2±12.7	0.2±8.5			

Mean±SD

SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, γ-glutamyltransferase

*$0.01 < P < 0.05$, $^{\$}0.05 < P < 0.1$, compared with placebo group

[1]$n = 34$ for the placebo group and $n = 32$ for the combination group

[2]$n = 13$ for the placebo group and $n = 15$ for the combination group

[3]$n = 21$ for the placebo group and $n = 17$ for the combination group

were no interactions between intervention and time（**Table 4**）. In addition, the decrease in ALT level from baseline to week 8 was greater in the combination group than in the placebo group and the decreases in γ-GTP level from baseline to weeks 8 and 12 were significantly greater in the combination group than in the placebo group. However, for participants whose serum liver enzyme levels were in the sound range, there were no significant between-group differences in AST, ALT, and γ-GTP level changes（**Table 4**）.

4　Inflammatory markers in relation to liver status

The changes in inflammatory marker levels from baseline to week 12 are shown in **Table 5**. Between the placebo and the combination groups, there were no significant differences in the changes of all inflammatory marker levels. To assess the relation between changes in inflammatory markers and liver states, participants were stratified into two groups: those with improved liver status（AST or ALT level had decreased at week 12 from baseline）and those without improved liver status（AST and ALT levels were maintained or had increased at week 12 from baseline）. The decreases in CRP, SAA, and IL-6 levels from baseline to week 12 were significantly greater in the improved group than in the unimproved group（**Table 6**）.

Table 5　Changes in inflammatory markers from baseline to week 12

	CRP（mg/dL）	SAA（μg/mL）	IL-6（pg/mL）	TNF-α（pg/mL）	PGE2（pg/mL）
Placebo	0.11±0.71	6.2±34.3	−0.08±1.84	−0.19±0.27	−116±304
Combination	−0.03±0.35	1.0±17.2	−0.34±1.82	−0.12±0.21	−114±236

Mean±SD
SD, standard deviation; CRP, C-reactive protein; SAA, serum amyloid protein A; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α ; PGE2, prostaglandin E2
n=36 for the placebo group and n=33 for the combination group

Table 6　Changes in inflammatory markers from baseline to week 12 stratified by liver status

	CRP（mg/dL）	SAA（μg/mL）	IL-6（pg/mL）
All participants[1]			
Improved	−0.11±0.35	−2.88±9.7	−0.77±1.97
Unimproved	0.02±0.12*	0.34±3.47*	−0.04±0.62*
Combination[2]			
Improved	−0.23±0.55	−5.74±15.6	−1.42±3.12
Unimproved	0.04±0.14$	1.14±3.91$	−0.01±0.36$
Placebo[3]			
Improved	−0.05±0.12	−1.28±3.15	−0.41±0.70
Unimproved	0.00±0.09	−0.35±2.96	−0.07±0.79

Mean±SD
SD, standard deviation; CRP, C-reactive protein; SAA, serum amyloid protein A; IL-6, interleukin-6
*0.01＜P＜0.05, $0.05＜$P$＜0.1, compared with improved group
[1]n=28 for improved and n=39 for unimproved（two participants were outliers because of extreme changes in SAA levels）
[2]n=14 for improved and n=18 for unimproved
[3]n=14 for improved and n=21 for unimproved

Although there was no difference in changes in CRP, SAA, and IL-6 levels between the improved group and the unimproved group for the placebo group, the improved group showed greater decreases in these levels than the unimproved group for the combination group（**Table 6**）.

5　POMS scores

The changes in POMS scores from baseline to each time point are shown in **Table 7**. The decrease in Tension-Anxiety score was greater in the combination group than in the placebo group and the decrease in fatigue score was significantly greater in the combination group than in the placebo group over the study period, but there were no interactions between intervention and time. In addition, the decrease in Tension-Anxiety score from baseline to week 8 was greater in the combination group than in the placebo group. The decrease in fatigue score from baseline to week 4 was significantly greater in the combination group than in the placebo group and the decrease from baseline to week 12 was greater in the combination group than in the placebo group.

6　Safety assessment

Forty-two adverse events（AEs）were recorded during the study. There were 17 AEs in the placebo group: backache（2）, cold symptoms（10）, elbow pain（1）, fatigue（1）, hemolytic streptococcal infection（1）, itch and eczema（1）, and pollinosis（1）. There were 25 AEs in the combination group: abdominal pain（1）, abnormal change of uric acid level（1）, backache（1）, cold symptoms（8）, sprain（1）, eye itch（1）, fatigue（1）, Ménière's disease（1）, possibility of herpes zoster（1）, rhinitis（1）, runny nose（1）, skin inflammation（1）, stomatitis（2）, toothache（2）, urinary occult blood（1）, and ventricular fibrillation（1）. Ventricular fibrillation was judged to be severe; 6 AEs（backache（1）, cold symptoms（1）, fatigue（1）, elbow pain（1）, abnormal change of uric acid level（1）, and urinary occult blood（1））were judged to be mild; and all the other AEs were judged to be moderate. The possibility of herpes

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

	Week 4）	Week 8	Week 12	*P*-value		
				Intervention	Time	Interaction
Tension-Anxiety						
Placebo	1.7±7.3	0.6±6.3	−0.5±6.0	0.064	0.18	0.76
Combination	0.1±4.9	−1.8±5.2$^{\$}$	−1.3±6.3			
Depression-Dejection						
Placebo	1.1±5.2	−0.1±5.3	−0.3±4.6	0.27	0.31	0.86
Combination	0.1±5.5	−1.2±5.2	−0.5±5.5			
Anger-Hostility						
Placebo	0.3±5.9	−0.6±5.2	−0.6±7.7	0.55	0.90	0.82
Combination	0.1±4.9	0.7±7.2	0.0±8.4			
Vigor						
Placebo	0.7±7.5	1.0±6.4	0.2±5.4	0.63	0.56	0.90
Combination	−0.1±6.8	1.2±7.7	−0.5±7.3			
Fatigue						
Placebo	3.5±6.4	2.3±7.3	1.2±6.5	0.0070	0.11	0.73
Combination	0.5±4.9*	0.8±5.5	−1.3±4.9$^{\$}$			
Confusion						
Placebo	1.8±7.5	0.3±5.8	−0.4±6.5	0.75	0.21	0.82
Combination	1.4±5.3	1.2±5.7	0.0±5.6			
Total mood disturbance						
Placebo	8±25	1±23	−1±23	0.23	0.14	0.90
Combination	2±16	−2±20	−3±19			

Mean±SD
SD, standard deviation
$^{*}0.01<P<0.05$, $^{\$}0.05<P<0.1$, compared with placebo group
$n=36$ for the placebo group and $n=33$ for the combination group

zoster was judged to be probably unrelated to the dietary intervention, and all the other AEs, including the severe AE（ventricular fibrillation）, were judged to be unrelated to the dietary intervention by a physician.

DISCUSSION

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, inflammatory markers, and mood states in healthy participants, a randomized, double-blind, placebo-controlled clinical trial was conducted. Throughout the intervention period, AST, ALT, and γ-GTP levels significantly decreased in the combination group compared with the placebo group for participants whose serum liver enzyme levels were in the borderline range. Serum liver enzymes such as ALT are often used as markers of hepatic function and increases in these enzymes reflect liver damage.[4,24,25] Even within the normal serum liver enzyme range, higher ALT levels are correlated with a greater cumulative incidence rate of diabetes.[26] 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.

We have recently shown that the intake of the combination decreases AST and ALT levels, but not γ-GTP level, in participants with borderline serum liver enzyme levels.[19] In this study, we showed that the combination was effective in decreasing γ-GTP, AST, and ALT levels in participants with borderline serum liver enzyme levels. There are considerable differences in initial γ-GTP levels between participants with borderline serum liver enzyme levels in the combination group in this study（49.3±22.0 U/L）and those in the previous study（36.8±17.4 U/L）. It is possible that the combination effectively decreases relatively high levels, but not low levels, of γ-GTP. It has been reported that there are significant positive correlation between ALT and γ-GTP and carotid intima-media thickness in patients with NAFLD.[27] Taken together, the combination might have the potential to decrease serum γ-GTP level in addition to decreasing AST and ALT levels.

Elevation of serum ALT level is associated with

higher CRP concentration[28] and IL-6 and CRP are increased in NAFLD or NASH patients.[7-9] Therefore, systemic inflammation is thought to be one of the exacerbating factors for NAFLD and NASH.[6] To assess the relation between changes in inflammatory markers and changes in liver enzyme levels, participants were stratified according to changes in liver enzyme levels（**Table 6**）. Analysis showed that the decreases in inflammatory markers such as CRP, SAA, and IL-6 were significantly greater in the improved group than in the unimproved group. This suggests that the decreases in inflammatory markers contributed to the improvement of liver enzyme levels. However, the placebo group did not show this pattern of changes, suggesting that the contribution of inflammatory marker decreases to the improvement of liver enzyme levels was more prominent in the combination group. Water extract of *C. longa*[13,14] and curcumin[17] have anti-inflammatory properties. Such anti-inflammatory agents can decrease serum liver enzyme levels and improve liver diseases.[29,30] It is possible that the combination decreased serum liver enzyme levels by suppressing liver and/or systemic inflammation.

Bisacurone is one of the bioactive compounds of *C. longa*[31] and has anti-inflammatory effects, such as inhibition of vascular cell adhesion molecule expression.[32] Hot water extract of *C. longa* also inhibits vascular cell adhesion molecule expression in endothelial cells through inhibiting the signaling of the protein complex NF-κB, suggesting that bisacurone is a potential active ingredient of hot water extract of *C. longa*.[13] Moreover, hot water extract of *C. longa* and bisacurone inhibits alcohol-induced liver injury in mice by suppressing oxidative stress and inflammation.[16] This mechanism may explain the improvement in liver function found in the present study from daily intake of hot water extract of *C. longa*, which contained 400 μg bisacurone.

The analysis of the POMS scores indicated that intake of the combination can improve negative emotional states, especially fatigue. Research suggests that negative emotional states such as fatigue or depression are related to inflammation in the brain[21] or the whole body.[20] Meta-analytic studies have reviewed the association of depression with serum inflammatory markers such as CRP and IL-6.[33,34] In this study, we assumed that the combination suppressed negative emotional states by inhibiting inflammation in the liver, brain, or the whole body through its anti-inflammatory effects, but there were no significant correlations between serum inflammatory markers and POMS scores（data not shown）. It is likely that we could not detect a relationship between inflammation and emotional states because the participants in this study had healthy emotional states; their inflammation levels were probably much lower than those of patients or animals in the above-mentioned studies. More studies are needed to investigate the effect of the combination on participants with more severe negative emotional states, such as clinically depressed patients, to assess its usefulness for such conditions.

CONCLUSIONS

We showed that a combination of hot water extract of *Curcuma longa* and curcumin significantly decreased borderline serum liver enzyme levels, possibly through the suppression of systemic inflammation. In addition, the combination significantly decreased POMS fatigue scores. The combination may be useful for the prophylaxis or treatment of borderline hepatitis and negative emotional states.

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