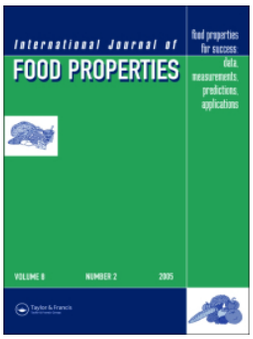

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Efficacy and anti-inflammatory properties of low-molecular-weight fucoidan in patients with atopic dermatitis: a randomized double-blinded placebo-controlled trial

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ABSTRACT

Atopic dermatitis (AD) is a chronic recurrent inflammatory skin disease. Fucoidans are reportedly effective in treating AD; however, their clinical efficacy requires further exploration. This study aimed to investigate the clinical efficacy of low-molecular-weight fucoidan (LMF) supplementation in patients with AD and reveal the underlying mechanism of its effects; this is a randomized, double-blind, placebo-controlled trial. Participants were randomly assigned to a study or control group to receive conventional AD therapy with oral supplementation of either LMF or placebo for 12 weeks. Symptom severity was measured by the SCORing Atopic Dermatitis (SCORAD) index. Each participant used a diary to record daily medication use. Blood samples were collected at three time points for assessing AD-related cytokines, immunoglobulin E (IgE), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood cell (WBC) count, the percentage of eosinophils, and biochemical profiles of hepatic and renal functions. The study group showed significant symptom relief, whereas the control group showed no significant improvements. The frequency of steroid ointment application significantly decreased, and the frequency of oral antihistamine use decreased in the study group, whereas no significant changes were observed in the control group. The AD-related immune parameters serum IgE, eosinophils, CD8+ T cells, interferon-γ (IFN-γ), ESR, and CRP significantly decreased in the study group but not the control group. There were no severe adverse events in either group. This study is the first to demonstrate the effectiveness and safety of LMF as a supplemental therapy for patients with AD via its anti-inflammatory activity.

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Low-molecular-weight fucoidan; Atopic dermatitis; SCORAD; inflammation; IFN-γ

Introduction

Atopic dermatitis (AD) is a common chronic recurrent inflammatory skin disorder characterized by skin hypersensitivity, intense itching, and eczema.[1] The prevalence of AD in Taiwan is 6.7%.[2] The clinical manifestations of AD include erythematous patches with exudation, blistering, and crusting at

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early stages and scaling, fissuring (cracking), and lichenification (thickening) at later stages.[3] Although AD can manifest at any point in life, it most often develops during childhood.[1] Patients with AD risk developing allergic disorders, such as allergic rhinitis and asthma.[4] AD can affect health-related quality of life; the higher the AD severity, the worse the health-related quality of life.[5] Moreover, AD substantially negatively impacts work productivity and daily activities. Therefore, more effective AD treatments are needed to alleviate the burden of the disease on the quality of life and work productivity of patients with AD.[6,7]

The pathogenesis of AD involves a complex interaction between genetic and environmental factors, which induces epidermal barrier dysfunction and immune dysregulation, primarily involving T helper (Th) 2 cell-mediated inflammation pathways, itching, and the skin microbiome.[3,8,9] AD management includes regular use of emollients, antihistamines, topical corticosteroids, topical calcineurin inhibitors, systemic immunosuppressive agents, phototherapy, and monoclonal antibody inhibitors.[10,11] However, there is little conclusive evidence on the effectiveness of H1 antihistamines in treating AD.[12] Moreover, patient adherence to corticosteroids and immunosuppressants is poor due to adverse effects concerns.[13,14] Dupilumab, an anti-interleukin (IL)-4 Rα monoclonal antibody that inhibits IL-4 and IL-13 signaling, is a new treatment for AD; however, its use is still limited because of its high cost.[15] Furthermore, in a population-based national health insurance database analysis, AD was found to impose significant healthcare costs, with an estimated total cost burden of NT$3.61 billion in Taiwan in 2017.[16] Therefore, new therapeutic agents for AD that ensure effectiveness, safety, and low costs are urgently needed.

Seaweeds have multiple biological activities. Fucoidans, one of the key polysaccharides in brown seaweeds, have been intensively studied over the past few years for their possible therapeutic potential. Depending on the extraction method, the molecular weight of fucoidans can range from 20,000 to 200,000 Da or from 400 to 5,000 Da, with the latter range being referred to as low-molecular-weight fucoidan (LMF).[17] Fucoidans have been reported to have various pharmacological effects, including anti-cancer, anti-viral, anti-inflammatory, immunomodulatory, anti-pathogen adhesion/infection, and antidiabetic activities and reduce *Helicobacter pylori* abundance.[18–20] The manufacturer's recommendations only specify a few contraindications, which include allergies to fucoidan, individuals with thyroid disease, and pregnant women. A previous study reported that fucoidan could suppress immunoglobulin E (IgE) induction in peripheral blood mononuclear cells derived from patients with AD.[21] In addition, a pilot study has observed that topical application of fucoidan could mitigate AD symptoms in a dinitrochlorobenzene (DNCB)-induced mouse model by suppressing the Th2 cell-mediated immune response.[22] Moreover, in a study using *in vitro* and *in vivo* AD models, fucoidan effectively inhibited mast cell degranulation. In addition, it decreased the levels of IL-4 and histamine, resulting *in vivo* systemic improvements, including suppressed epidermal hyperplasia, improved wound healing, reduced eosinophil infiltration, and decreased expression levels of AD-associated cytokines.[23] However, the clinical efficacy and safety of fucoidans are not yet fully understood.

In this study, we conducted a randomized, double-blind, placebo-controlled trial to investigate the clinical effects and safety of LMF as a supplemental therapy in managing AD. Moreover, we investigated the underlying mechanisms of LMF in patients with AD.

Materials and methods

Study design

This randomized, double-blind, placebo-controlled trial was conducted between March 2017 and July 2020 at Chang Gung Memorial Hospital (CGMH) in Taoyuan, Taiwan. Dermatologists and traditional Chinese medicine doctors enrolled the patients after considering the inclusion and exclusion criteria. Participants were randomly assigned in a 2:1 ratio to receive conventional AD therapy (including steroid ointment application and oral

antihistamines according to clinician's instructions) with oral supplementation of either LMF or placebo for 12 consecutive weeks. In addition, they were taught to reduce the frequency of steroid ointment application and oral antihistamine use if tolerable pruritus and stable rash areas.

Four visits to the outpatient clinic to assess symptom severity were arranged for each participant with the following schedule: week zero (before supplementation with LMF/placebo), week six (six weeks after supplementation with LMF/placebo), week 12 (12 weeks after supplementation with LMF/placebo, end of supplementation), and week 16 (end of the trial). The severity of AD symptoms was evaluated by a doctor using the SCORing Atopic Dermatitis (SCORAD) index for each participant at weeks zero, six, 12, and 16. Each participant used a diary to record daily medication use. Blood samples were collected at three time points (weeks zero, 12, and 16) for the assessment of AD-related cytokine levels, IgE levels, C-reactive protein (CRP) levels, erythrocyte sedimentation rate (ESR), white blood cell (WBC) count, eosinophil percentage, and biochemical profiles of hepatic and renal function. All participants provided written informed consent. This study was conducted in accordance with the Declaration of Helsinki. The trial protocol was approved by the Institutional Review Board (IRB) of the Chang-Gung Medical Foundation (IRB No. 201601520A3C601) and performed in accordance with the recommendations of the guidelines for clinical trials of the same committee. The study was registered at https://www.isrctn.com/ (Identifier ISRCTN90251749).

Participants

The inclusion criteria were as follows: (1) male and female patients aged between four and 60 years; (2) evidence of itchy skin (or parental report of scratching or rubbing), combined with three or more of the following: a) History of itchiness in skin creases (e.g., front of elbows, back of knees, front of ankles, and areas around the neck or eyes); b) History of asthma or hay fever; c) History of generally dry skin in the past year; d) Onset in children under two years of age; and e) Visible flexural dermatitis; (3) SCORAD index ≥ 16 points; and (4) voluntarily agreeing to participate and providing informed consent.

The exclusion criteria were as follows: (1) Other eczema disorders, such as contact or seborrheic dermatitis, assessed by a dermatologist; (2) Other dermatological diseases related to skin pruritus, assessed by a dermatologist; (3) Patients using oral/intravenous steroids, leukotriene antagonists, immunosuppressants, systemic photochemotherapy, immunotherapy, allergen-specific immunotherapy, or Chinese herbal medicine within one month before enrollment; (4) Patient unable to take the trial supplements as scheduled or cooperate in filling out the questionnaire and taking a blood test; (5) Patients with a history of allergy to fucoidan; (6) Patients with a history of hyperthyroidism; (7) Patients suffering from serious infection requiring hospitalization (e.g., pneumonia, cellulitis, or sepsis), assessed by a clinician; (8) Severe organ dysfunction (e.g., heart failure, liver failure, liver cirrhosis, or renal failure [eGFR <60 mL/min/1.73 m^2]), assessed by a clinician; and (9) Pregnant women or those preparing for pregnancy, and those in lactation.

Material preparation

The LMF powders in this trial were derived from *Sargassum hemiphyllum*, manufactured by Hi-Q Marine Biotech International Ltd. (Taiwan). They were qualified as a Symbol of National Quality products in Taiwan. LMF was produced through enzymatic hydrolysis of the original fucoidan. The characteristics of LMF were an average molecular weight of 0.8 kDa (92.1%), fucose content of 210.9 ± 3.3 μmol/g, and sulfate content of 38.9 ± 0.4% (w/w).[24] The extraction method was performed as previously described.[25] Each capsule contained 500 mg of LMF in the study group, whereas each capsule contained 500 mg of microcrystalline cellulose as

a placebo in the control group. The appearance of the capsules in the study and control groups was the same.

Intervention

Participants in the study group took LMF capsules orally twice daily before meals for 12 weeks; those in the control group took placebo capsules. The dosage of supplementation for participants was determined according to their body weight. The dosage for body weight over 40 kg was 4.0 g/day (four capsules twice daily), between 20 and 40 kg was 3.0 g/day (three capsules twice daily), < 20 kg was 2.0 g/day (two capsules twice daily), and <10 kg was 1.0 g/day (one capsule twice daily).

Randomization and masking

Eligible participants were randomly assigned to the study or control groups in a 2:1 ratio. After signing a written informed consent form, the researchers assigned each participant a project number. The randomization code list was generated using a permuted block randomization method with a block size of three by Hi-Q Marine Biotech International Ltd. and was unblinded after the trial was completed. The manufacturer labeled the study capsules with identical appearance according to the generated randomization code list. The pharmacy of CGMH was then provided with these study capsules in consecutive numbers randomized as LMF and placebo. The pharmacists dispensed the study capsules to the participants according to their project number. The participants, pharmacists, outcome assessors, and investigators were blinded to the randomization outcomes.

Outcome measurement

The primary outcome of this study was the symptom severity of AD, which was assessed using the SCORAD index, an evaluation index often used to score AD severity. Investigators and dermatologists scored the extent and intensity of AD lesions as objective indicators and pruritus and sleeplessness as subjective indicators.[26,27] The secondary outcomes were the changes in the frequency of Western medicine use (including steroid ointment application and oral antihistamine usage) and the immune parameters related to AD, including IgE levels, WBC count, the percentage of eosinophils, ESR, CRP, IL-4, IL-10, IL-12, IL-13, interferon-γ (IFN-γ), and the percentage of $CD4^+$ and $CD8^+$ T lymphocytes. In addition, participants were asked to keep a daily diary during the trial to record their daily use of trial medication and Western medicine, if needed.

Blood samples collected from participants were sent to the Department of Laboratory Medicine at CGMH to test for IgE levels, WBC count, eosinophil percentage, ESR, CRP levels, aspartate aminotransferase (AST) levels, alanine aminotransferase (ALT) levels, and creatinine levels. In addition, samples were labeled with antibodies against CD4 and CD8 (BD Biosciences, San Jose, CA, USA) to examine lymphocyte subsets. The samples were analyzed by flow cytometry using the appropriate isotype controls. Cytometric analyses were performed using a BD FACSCanto™ II Clinical Flow Cytometry System equipped with FASDiva Software (BD Biosciences). In addition, serum cytokine levels (IL-4, IL-10, IL-12, IL-13, and IFN-γ) were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits according to the manufacturer's instructions (Quantikine; R&D Systems, Minneapolis, MN, USA).

Safety and adverse events monitoring

For safety assessment, laboratory tests, including complete blood count and biochemical profiles of hepatic and renal function (AST, ALT, and creatinine), were performed on weeks zero, 12 (end of

LMF/placebo supplementation), and 16 (end of the trial). The participants were encouraged to report any symptoms or discomfort to the project manager at any time throughout the trial. All suspected events were closely monitored during the trial period.

Statistical analysis

Data are presented as the mean ± standard deviation. SPSS version 29.0.0.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. The efficacy analysis was based on a per-protocol analysis set of 82 participants (54 in the study group and 28 in the control group). A paired sample *t*-test was used to analyze the changes in the SCORAD index, steroid ointment application, and oral antihistamine use between weeks six, 12, 16, and baseline (week zero). The Wilcoxon signed-rank test was applied to analyze the changes in the parameters from their baseline values. A two-tailed $p < .05$ was considered statistically significant.

Results

Baseline characteristics

From March 2017 to July 2020, 98 volunteers underwent screening, of whom seven were excluded because they did not meet the inclusion criteria. Eligible participants ($n = 91$) were randomly assigned to either the study ($n = 60$) or the control group ($n = 31$) at a 2:1 ratio. Figure 1 shows a detailed flow diagram of the enrollment. Of these participants, nine could not complete the 16-week trial (six in the study group and three in the control group), two were lost during follow-up

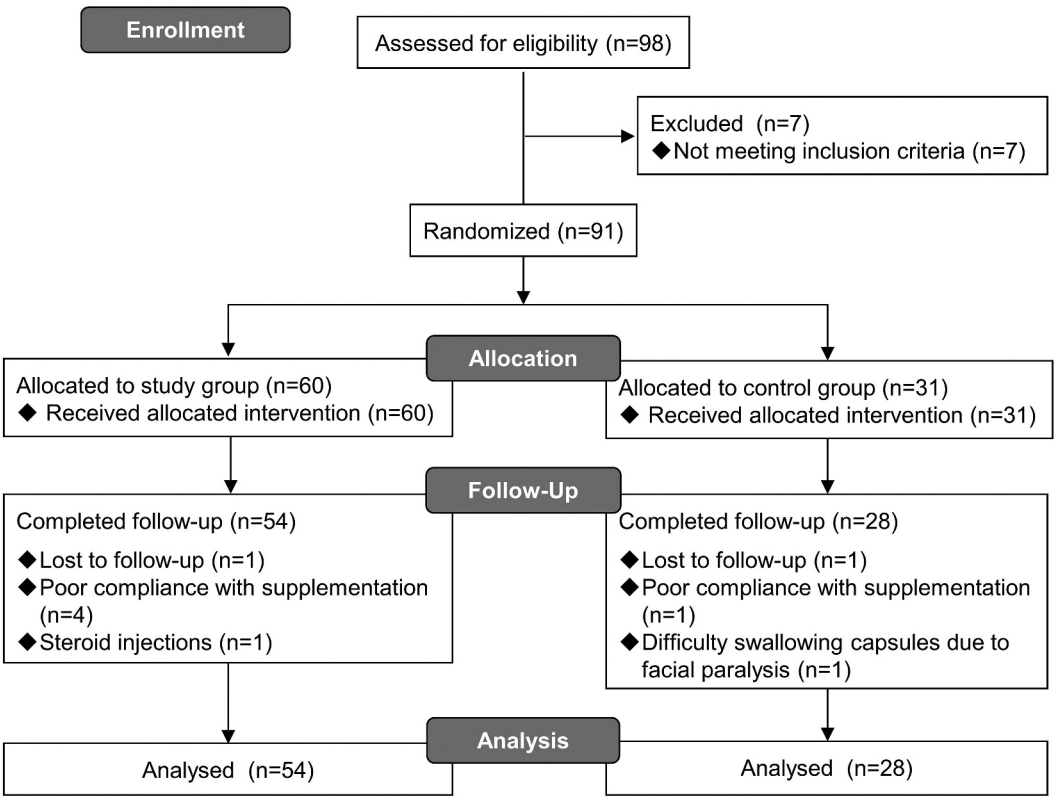

Figure 1. CONSORT flow diagram of the study.

Table 1. Demographic and baseline characteristics of participants.

Characteristics	Study group ($n = 54$)	Control group ($n = 28$)	p-value
Age group (years)	***N***	***N***	0.946
4–10	14	8	
11–20	13	9	
21–30	12	4	
31–40	8	4	
41–50	5	2	
51–60	2	1	
Sex, men/women	***N***	***N***	
	27/27	11/17	0.356
SCORAD Index	Mean ± SD	Mean ± SD	
Extent of lesion	22.52 ± 21.52	17.61 ± 13.99	0.278
Intensity of lesion	7.03 ± 2.49	6.70 ± 2.40	0.565
Subjective symptoms	10.66 ± 4.90	10.63 ± 4.65	0.979
Total score	39.78 ± 14.28	37.61 ± 13.20	0.507
Frequency of Western medication use	Mean ± SD	Mean ± SD	
Steroid ointment application (days/week)	3.75 ± 3.49	2.93 ± 3.46	0.317
Oral antihistamine (days/week)	1.91 ± 3.11	1.04 ± 2.53	0.185
Serologic Markers	Mean ± SD	Mean ± SD	
IgE (KU/L)	2216.6 ± 401.9	2274.2 ± 528.9	0.715
Eosinophil (%)	6.04 ± 0.76	6.88 ± 0.94	0.083
WBC (10^3/μL)	7.64 ± 0.29	8.26 ± 0.27	0.275
ESR (mm/h)	7.66 ± 0.71	8.18 ± 0.92	0.464
CRP (mg/L)	1.24 ± 0.35	1.08 ± 0.30	0.616
Cr (mg/dL)	0.62 ± 0.03	0.59 ± 0.03	0.966
AST (U/L)	27.74 ± 1.81	24.96 ± 1.33	0.220
ALT (U/L)	21.15 ± 2.08	16.79 ± 1.75	0.288

Statistics were evaluated using the chi-squared test of independence for categorical variables, independent samples *t*-test for SCORAD index, and Wilcoxon signed-rank test for serologic markers. ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine; CRP, C-reactive protein; ESR, erythrocytes sedimentation rate; IgE, immunoglobulin E; N, numbers; SCORAD, SCORing Atopic Dermatitis; SD, standard deviation; WBC, white blood cell.

(one in the study group and one in the control group), five had poor supplementation compliance (four in the study group and one in the control group), one self-administered steroid injections at another hospital (in the study group), and one had difficulty swallowing capsules due to facial paralysis (in the control group). Ultimately, a per-protocol analysis was performed with 82 participants, including 54 in the study group and 28 in the control group. Table 1 presents the demographic and baseline characteristics of the 82 patients who completed the trial. No significant differences were observed between the two groups in terms of patient age, sex, initial SCORAD index, frequency of Western medicine use (including steroid ointment application and oral antihistamine use), and serologic markers.

Primary outcome: effect on symptom severity after supplementation with LMF for three months measured using the SCORAD Index

Figure 2 and Table 2 summarize the changes in the symptom severity of AD after supplementation with LMF for three months. Significant symptom relief was observed in the study group according to the SCORAD index. In the study group, the objective SCORAD index measuring the extent of the lesions was 22.52 ± 21.52 at baseline and significantly decreased to 17.74 ± 15.10 and 16.69 ± 15.33 at weeks six and 12, respectively ($p < .05$); Figure 2(a). The objective SCORAD index measuring the intensity of the lesions was 7.03 ± 2.49 at baseline and significantly decreased to 6.15 ± 2.49 and 5.78 ± 2.66 at weeks six and 12, respectively ($p < .01$); Figure 2(b). Furthermore, the subjective SCORAD index measuring

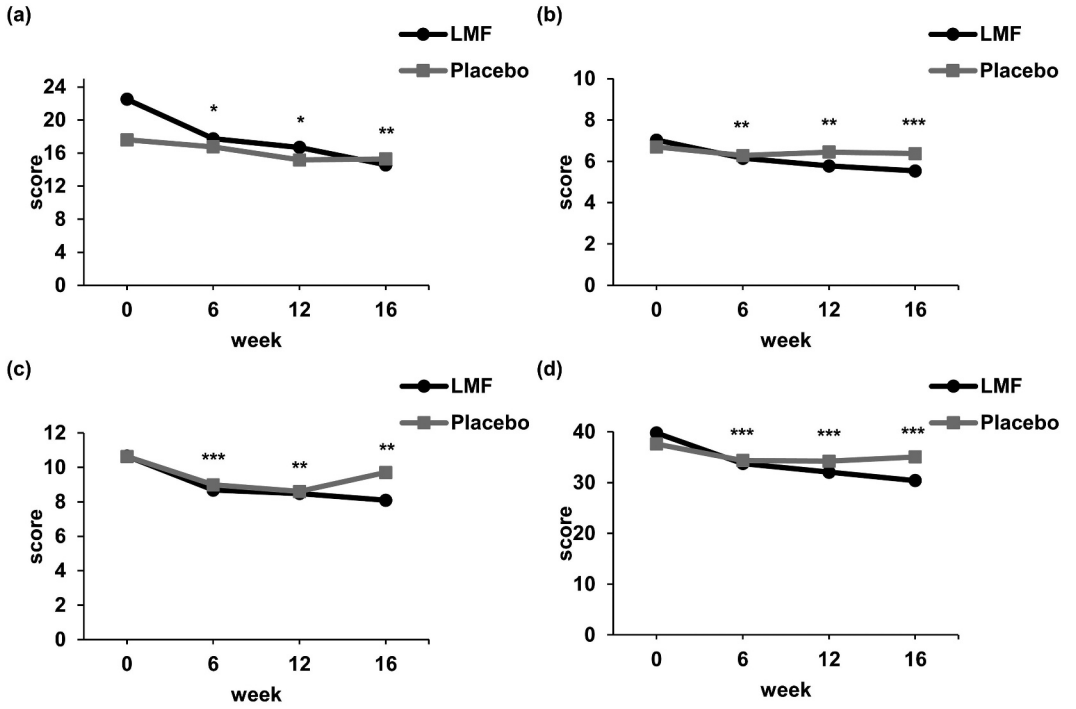

Figure 2. Evolution of the SCORAD index from baseline to weeks six, 12, and 16. (a) Lesion extent, (b) lesion intensity, (c) subjective symptoms, (d) total. *$p < .05$, **$p < .01$, ***$p < .001$ vs. baseline. LMF, low-molecular-weight fucoidan.

pruritus and sleeplessness symptoms was 10.66 ± 4.90 at baseline and significantly decreased to 8.69 ± 4.77 and 8.48 ± 5.33 at weeks six ($p < .001$) and 12 ($p < .01$); Figure 2(c), respectively. The total SCORAD index was 39.78 ± 14.28 at baseline and significantly decreased to 33.77 ± 13.48 and 32.06 ± 14.58 at weeks six ($p < .001$) and 12 ($p < .001$); Figure 2(d), respectively. The scores continued to decline significantly even after discontinuation of LMF for one month Figure 2(a–d). In the control group, the objective and subjective SCORAD indices did not significantly differ before and after placebo supplementation; the total SCORAD index decreased from 37.61 ± 13.20 to 34.35 ± 16.34 and 34.22 ± 14.95 at weeks six and 12, respectively, without obvious differences.

Secondary outcomes

Effect on steroid ointment application and oral antihistamine use

We summarized the changes in steroid ointment application and oral antihistamine use after supplementation with LMF for three months (Table 3). No significant differences were observed between the two groups for the frequency of Western medicine use (including steroid ointment application and oral antihistamine use) at baseline. However, the frequency of steroid ointment application was 3.75 ± 3.49 days/week at baseline and significantly decreased to 2.42 ± 2.85 days/week during weeks 1–6 ($p < .001$) and 2.16 ± 2.83 days/week during weeks 7–12 ($p < .001$) in the study group; Figure 3(a). Furthermore, this effect was preserved even after discontinuing LMF for a month (1.89 ± 2.86 days/week during weeks 13–16; $p < .001$). In addition, the frequency of oral antihistamine use in the study group decreased during weeks 1–6 and 7–12, compared with that of the baseline ($p = .074$ and 0.057, respectively); Figure 3(b). There was no significant difference in the frequency of steroid ointment application and oral antihistamine use before and after placebo supplementation in the control group.

Table 2. SCORAD index changes over time in the study and control groups.

	Study group ($n = 54$)				Control group ($n = 28$)			
	Week 0	Week 6	Week 12	Week 16	Week 0	Week 6	Week 12	Week 16
Extent of lesion	22.52 ± 21.52	17.74 ± 15.10 *	16.69 ± 15.33 *	14.57 ± 11.00 **	17.61 ± 13.99	16.75 ± 16.23	15.18 ± 14.53	15.29 ± 15.21
Intensity of lesion	7.03 ± 2.49	6.15 ± 2.49 **	5.78 ± 2.66 **	5.54 ± 2.41 ***	6.70 ± 2.40	6.29 ± 2.74	6.45 ± 2.51	6.38 ± 2.56
Subjective symptoms	10.66 ± 4.90	8.69 ± 4.77 ***	8.48 ± 5.33 **	8.10 ± 5.05 **	10.63 ± 4.65	8.99 ± 5.81	8.60 ± 5.34	9.71 ± 5.42
Total score	39.78 ± 14.28	33.77 ± 13.48 ***	32.06 ± 14.58 ***	30.41 ± 12.32 ***	37.61 ± 13.20	34.35 ± 16.34	34.22 ± 14.95	35.06 ± 14.83

The SCORAD index was used to assess the symptom severity of AD. The data are presented as mean ± standard deviation. *$p < .05$, **$p < .01$, ***$p < .001$ vs. baseline.

Table 3. Changes in the frequency of steroid ointment application and oral antihistamine use during the trial period in the study and control groups.

	Study group ($n = 54$)				Control group ($n = 28$)			
	Baseline	Week 1–6	Week 7–12	Week 13–16	Baseline	Week 1–6	Week 7–12	Week 13–16
Frequency of steroid ointment application (days/week)	3.75 ± 3.49	2.42 ± 2.85 ***	2.16 ± 2.83 ***	1.89 ± 2.86 ***	2.93 ± 3.46	1.89 ± 2.63	2.19 ± 2.84	2.31 ± 3.09
Frequency of oral antihistamine use (days/week)	1.91 ± 3.11	1.23 ± 2.21	1.06 ± 2.27	1.26 ± 2.46	1.04 ± 2.53	1.00 ± 2.00	1.19 ± 2.48	1.45 ± 2.58

The data are presented as mean ± standard deviation. *** $p < .001$ vs baseline.

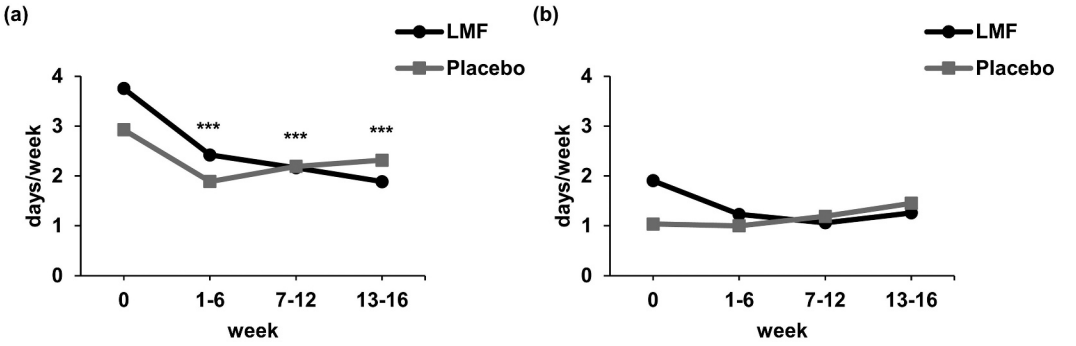

Figure 3. Changes in the frequency of (a) steroid ointment application and (b) oral antihistamine usage. *** $p < .001$ vs baseline. LMF, low-molecular-weight fucoidan.

Table 4. Changes in immune parameters related to AD.

	Study group ($n = 54$)			Control group ($n = 28$)		
Parameter	Week 0	Week 12	Week 16	Week 0	Week 12	Week 16
IgE (KU/L)	2216.6 ± 401.9	2090.2 ± 370.8	2019.6 ± 359.7#	2274.2 ± 528.9	2619.7 ± 633.7*	2716.6 ± 663.6
Eosinophil (%)	6.036 ± 0.764	5.181 ± 0.463	4.734 ± 0.372 *	6.882 ± 0.936	6.293 ± 0.638	6.600 ± 0.711
ESR (mm/h)	7.66 ± 0.71	6.78 ± 0.63	6.44 ± 0.61 *	8.18 ± 0.92	9.89 ± 1.55	7.89 ± 0.82
CRP (mg/L)	1.24 ± 0.35	0.77 ± 0.13	0.67 ± 0.12 *	1.08 ± 0.30	1.60 ± 0.51	1.60 ± 0.38**
WBC (10^3/μL)	7.638 ± 0.287	7.624 ± 0.287	7.476 ± 0.306	8.257 ± 0.269	8.246 ± 0.394	8.436 ± 0.436
IFN-γ (pg/mL)	3.499 ± 0.293	3.411 ± 0.343	2.741 ± 0.402 * #	3.700 ± 0.345	3.444 ± 0.404	3.068 ± 0.307
IL-4 (pg/mL)	0.068 ± 0.010	0.069 ± 0.006	0.077 ± 0.006	0.063 ± 0.008	0.086 ± 0.009	0.106 ± 0.018 *
IL-10 (pg/mL)	0.267 ± 0.052	0.248 ± 0.068	0.224 ± 0.057	0.154 ± 0.066	0.156 ± 0.084	0.113 ± 0.052
IL-12 (pg/mL)	0.252 ± 0.129	0.217 ± 0.126	0.214 ± 0.106	0.220 ± 0.103	0.215 ± 0.151	0.178 ± 0.092
IL-13 (pg/mL)	1.335 ± 0.066	1.356 ± 0.070	1.298 ± 0.064	1.475 ± 0.058	1.503 ± 0.064	1.461 ± 0.085
CD4+ T cells (%)	38.16 ± 1.55	38.75 ± 1.53	38.51 ± 1.46	35.44 ± 2.04	35.92 ± 1.93	36.25 ± 1.78
CD8+ T cells (%)	33.33 ± 1.05	32.00 ± 1.02	31.67 ± 1.01 *	33.16 ± 1.63	32.46 ± 1.22	31.09 ± 1.31 #

The data are presented as mean ± standard deviation (Wilcoxon signed-rank test). * $p < .05$, ** $p < .01$ vs. baseline. # $p < .05$ vs. week 12. IFN-γ, interferon-γ; IL, interleukin.

Effect on Serologic Markers Related to AD

Changes in the immune parameters related to AD are summarized in Table 4. A significant decrease in IgE levels, ESR, CRP levels, IFN-γ levels, eosinophil percentage, and CD8+ T cell number was observed in the study group after LMF supplementation, with total serum IgE levels continually decreasing from 2,216.6 ± 401.9 KU/L at baseline to 2,090.2 ± 370.8 KU/L and 2,019.6 ± 359.7 KU/L at weeks 12 and 16, respectively. A significant difference in IgE levels was observed in the within-group analysis (week 16 vs week 12; $p = .030$); Figure 4(a). However, in the control group, serum total IgE levels continually increased from 2,274.2 ± 528.9 KU/L at baseline to 2,619.7 ± 633.7 KU/L and 2,716.6 ± 663.6 KU/L at weeks 12 and 16, respectively. A significant increase in IgE levels was observed in the within-group analysis of the control group (week 12 vs baseline; $p = .032$); Figure 4(a). ESR significantly decreased in the study group from 7.66 ± 0.71 mm/h to 6.44 ± 0.61 mm/h over 16 weeks ($p = .028$), whereas no significant changes were observed in the control group; Figure 4(b). CRP levels in the study group significantly ($p = .049$) decreased from 1.24 ± 0.35 mg/L at baseline to 0.67 ± 0.12 mg/L at week 16, whereas they significantly ($p = .006$) increased in the control group from 1.08 ± 0.30 mg/L to 1.60 ± 0.38 mg/L; Figure 4(c). Furthermore, the percentage of eosinophils in the study group was 6.036 ± 0.764 at baseline and decreased to 5.181 ± 0.463 and 4.734 ± 0.372 at weeks 12 and 16, respectively. The within-group analysis revealed a significant decrease in the study group week 16 vs baseline ($p = .029$); Figure 4(d).

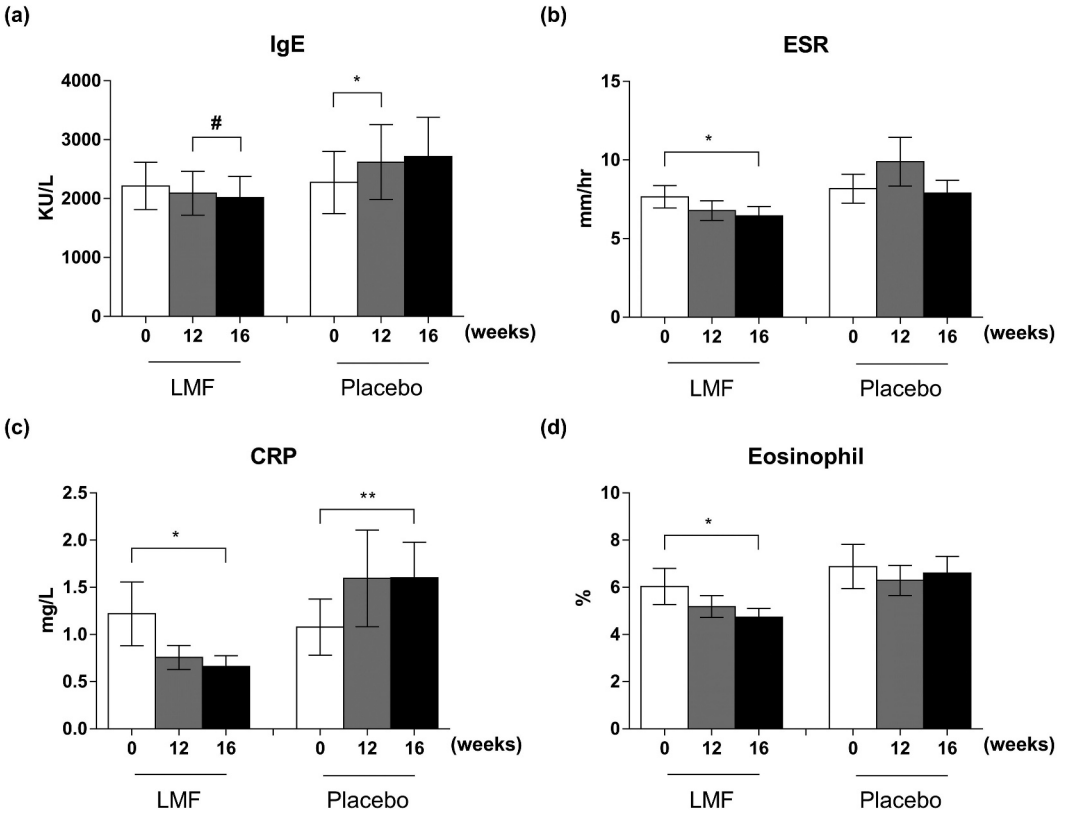

Figure 4. Changes in serum IgE levels, ESR, CRP levels, and eosinophil percentage. Data are expressed as mean ± standard deviation (Wilcoxon signed-rank test). * $p < .05$, ** $p < .01$ vs. baseline. # $p < .05$ vs. week 12. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IgE, immunoglobulin E.

In addition, although there were no significant within-group differences in the percentage of $CD4^+$ T cells at baseline, week 12, and week 16 in both groups; Figure 5(a), we observed a significant difference in the percentage of $CD8^+$ T cells in the within-group analysis of the study group (week 16 vs baseline; $p = .039$); Figure 5(b). At baseline, the percentage of CD8+ T cells in the study group was 33.33 ± 1.05 and decreased to 32.00 ± 1.02 and 31.67 ± 1.01 at weeks 12 and 16, respectively. Serum IFN-γ levels in the study group decreased from 3.499 ± 0.293 pg/mL at baseline to 3.411 ± 0.343 and 2.741 ± 0.402 pg/mL at weeks 12 and 16, respectively (week 16 vs baseline; $p = .027$). There were also significant differences in IFN-γ levels between weeks 16 and 12 ($p = .023$); Figure 5(c). The levels of cytokines, including IL-4, IL-10, IL-12, and IL-13, were not significantly different at baseline, week 12, and week 16 in both groups, except for the level of IL-4 in the control group, which showed a significant increase from 0.063 ± 0.008 pg/mL at baseline to 0.106 ± 0.018 pg/mL at week 16 ($p = .043$); Figure 5(d–g).

Safety evaluation

No severe adverse events were observed in either group during the trial period. None of the participants dropped out of the study because of the side effects of LMF. Hepatic and renal functions were all within the normal range in both groups before and after supplementation (Figure 6).

Discussion

To our knowledge, this clinical trial is the first randomized, double-blind, placebo-controlled clinical trial of fucoidan supplementation for managing AD. We revealed that the study group achieved significant

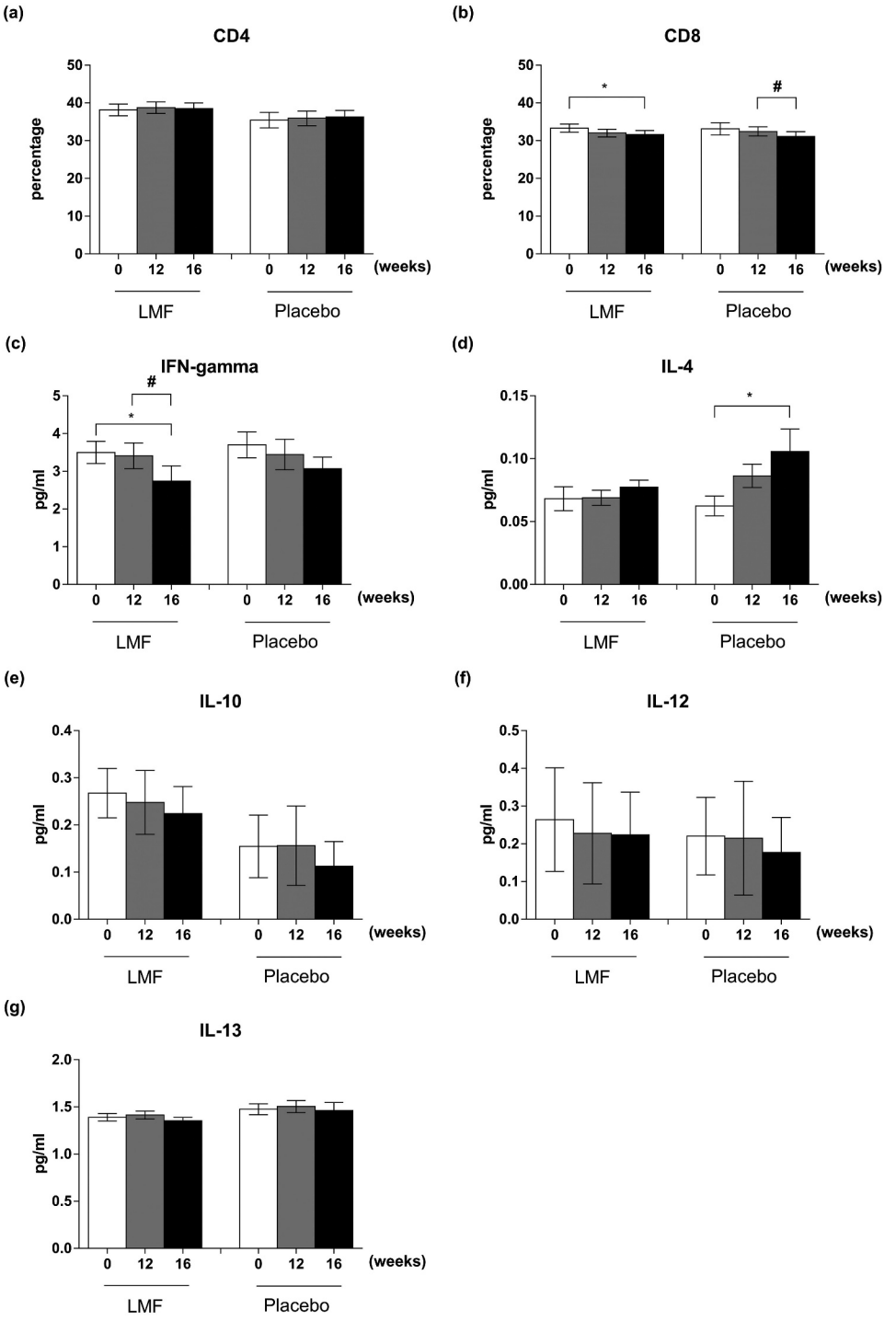

Figure 5. $CD4^+$ and $CD8^+$ T cell percentages and IFN-γ, IL-4, IL-10, IL-12, and IL-13 levels. Data are expressed as mean ± standard deviation (Wilcoxon signed-rank test). * $p < .05$ vs. baseline. # $p < .05$ vs. week 12. IFN-γ, interferon-γ; IL, interleukin.

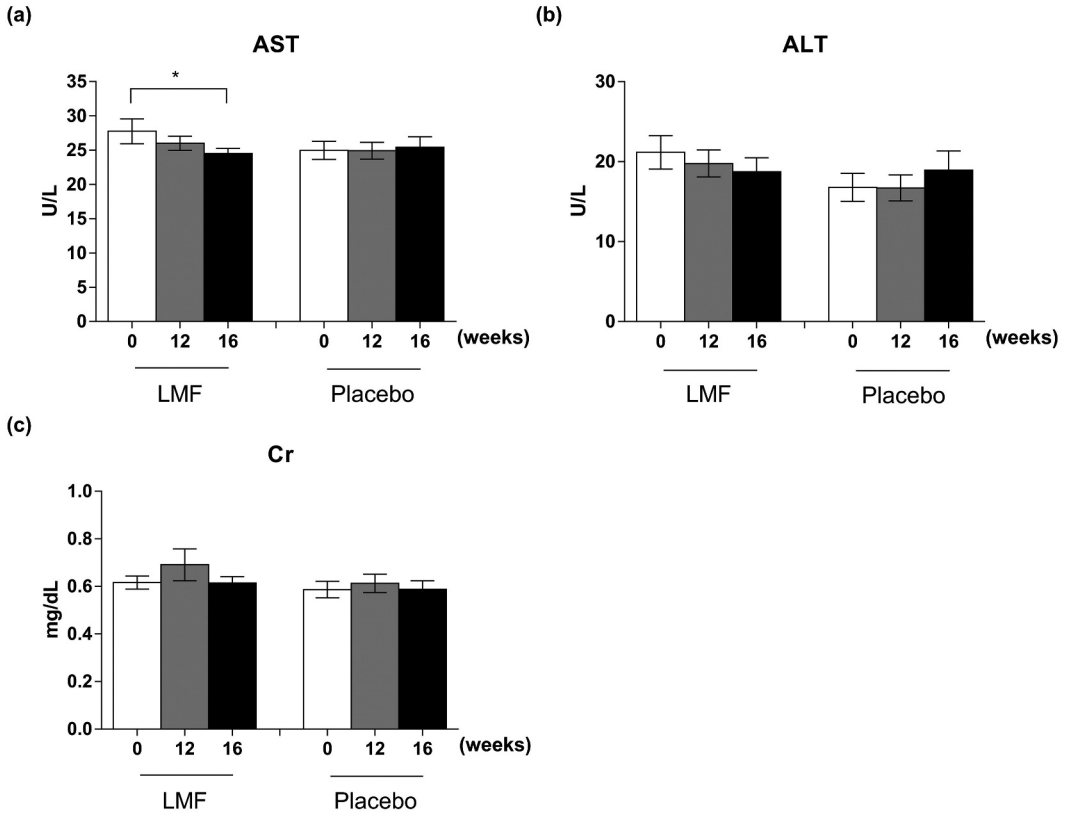

Figure 6. Safety Evaluation. Data were within the normal range before and after treatment in both groups. Values are expressed as mean ± standard deviation (Wilcoxon signed-rank test).

improvements in symptom severity as early as week six, and the effect persisted for four weeks after stopping supplementation. In addition, the total SCORAD index significantly decreased after six weeks of LMF supplementation and continued to decline after 12 weeks of LMF supplementation; this is consistent with a previous animal study that demonstrated that oral administration of fucoidan from *Cladosiphon okamuranus* (COP) lowered the SCORAD index in a DNCB-induced mouse model.[23] We also analyzed the objective SCORAD index, which measures the extent and intensity of dermal lesions, and the subjective SCORAD index, which measures pruritus and sleep disorders, both of which decreased after LMF supplementation, consistent with the total SCORAD index. More importantly, the efficacy of LMF in improving symptoms increased during the follow-up phase.

Topical corticosteroids and oral antihistamines are generally used for AD patients to control pruritus and allergic complications.[28,29] In the present study, to further evaluate the clinical efficacy of LMF, we measured the frequency of Western medicine use, including steroid ointment application and oral antihistamine use during the trial period. The baseline frequency of Western medication use did not reach a significant difference between the study and control groups (Table 1). However, we observed a significant decrease in the frequency of steroid ointment application during six weeks of LMF supplementation compared with that of the baseline, which persisted during weeks 7–12 of LMF supplementation and a month after LMF discontinuation. Furthermore, although statistical significance was not achieved in the frequency of oral antihistamine use in the study group, LMF supplementation tended to lead to reduced usage of oral antihistamines. The results indicate that LMF, a marketed health supplement, demonstrates efficacy when used as an adjunctive therapy in the treatment of

patients with AD, resulting in a reduction in the need for conventional AD therapy. Additionally, the cost of LMF falls within an affordable range for the majority of patients.

Changes in AD-related serum immunologic markers may help reveal the potential mechanisms of LMF. Our study demonstrated a continuous downward trend of IgE, percentage of eosinophils, $CD8^+$ T cells, IFN-γ, ESR, and CRP in the study group after 12 weeks of LMF supplementation and a month after LMF withdrawal. Although we did not observe differences in the levels of Th2-related cytokines, such as IL-4, IL-13, and IL-10, after supplementation with LMF, elevated IL-4 serum levels were observed in the control group, suggesting that LMF may ameliorate the progressive immune dysfunction associated with AD. In BALB/c mice, the administration of fucoidan for six weeks decreased IL-4, IL-5, and IgE production.[30] Moreover, fucoidan reduced the levels of IL-4 and histamine *in vitro* and the levels of serum IgE and AD-associated cytokines, including IL-4, IL-5, IL-6, IL-13, IL-22, TSLP, TNF-α, IFN-γ, and IL-1β, in DNCB-induced mice *in vivo*.[23] Therefore, our results are consistent with previous reports.

AD is a biphasic T-cell-mediated inflammatory disease, wherein a Th2 response causes the acute phase and a Th1 response leads to the chronic phase.[31] Furthermore, additional pathways implicated in the pathogenesis of AD have been reported, including cytokines associated with Th17 and Th22 responses.[32,33] AD outbreaks result from IgE-induced type I hypersensitivity, stimulating Th2 cell differentiation. Th2 cells produce IL-4, IL-5, IL-13, and IL-31, which inhibit the expression of filaggrin, loricrin, and involucrin, thereby establishing a relationship between immune dysfunction and disruption of skin barrier function in AD.[1,32,34] The inflammatory cytokines IL-4, IL-13, and IL-31 can directly activate pruriceptors in mice and humans and mediate scratching behavior, leading to further skin barrier damage and entry into the itch-scratch cycle.[35] Moreover, IL-4 initiates T-cell differentiation toward the Th2 subtype and subsequently produces additional IL-4 in a positive feedback loop, and IL-4 induces isotype class switching to IgE in B cells. Th2 cells induce B-cell proliferation and the subsequent production of antibodies, resulting in high levels of serum IgE.[36] In AD, the hallmark of type 2 inflammation is eosinophilia and/or high serum IgE levels.[37] Regarding the role of IL-10 in AD, it is controversial based on current research.[38] Report indicated that decreased serum IL-10 levels could be detected in AD patients, particularly in cases where there is higher activity or a severe progression of the condition.[39] Another report suggested that IL-10 have a suppressive effect on the chronic inflammation of AD patients.[40] However, the levels of IL-10 in biopsy specimens are upregulated, particularly in pruritic atopic skin lesions.[41,42] AD susceptibility can be linked to IL-10 gene polymorphisms. [43]

In contrast, increased expression levels of IL-12 and IFN-γ are characteristic of a Th1 immune response, which plays a role in the chronic stage of AD[31]; IFN-γ is highly expressed in chronic AD.[44] Furthermore, IFN-γ decreases the levels of ceramides and long-chain fatty acids, leading to epidermal dysfunction.[45] Additionally, $CD8^+$ T cells are potent producers of IFN-γ, IL-13, and IL-22 in AD, which may play an important role in epidermal thickening and skin barrier disruption.[46] Th17 and Th22 responses are also associated with AD. Th17 cells generate IL-17 and IL-22, whereas Th22 cells exclusively produce the latter cytokine. Compared to individuals with healthy skin, the expression of IL-17 is elevated in certain subgroups of AD, such as in Asians. However, this increase is not as pronounced as observed in other inflammatory skin conditions like psoriasis. Notably, there is a significant upregulation of IL-22 in AD skin, and this upregulation is closely associated with the severity of the disease.[32,47] Another study showed that IgG of AD patients can stimulate IL-17 and IL-10 production in infant intrathymic CD4 and CD8 cells.[48] Later, IgG from AD patients could activate non-atopic infant thymic invariant natural killer T (iNKT) cells to produce IL-4, IL-10 and IL-17 was described. Therefore, IgG may play a modulatory role in the development of AD was suggested.[49] Our results demonstrate that LMF supplementation may result in a sustained improvement in AD symptom severity by reducing the percentage of eosinophils and IgE levels as well as exerting anti-inflammatory activity by targeting Th1-related cytokines such as IFN-γ. To fully understand the immunomodulatory effects of LMF on patients with AD, further research is required.

This study had several limitations. First, there was only one LMF dose group, which limits the accurate evaluation of the effects of LMF. As LMF was categorized as a health supplement, our study followed the recommended standard dosage. Furthermore, given that LMF is not a pharmaceutical medication and is designed solely for supplementary purposes, we did not explore dosage escalation or reduction in our research. Further dose-response relationships with larger sample sizes are needed. Second, whether to reduce Western medication or not mainly depended on the patient's subjective judgment without strict criteria, such as the number of itching bouts and size of rash areas, which may partially affect the outcomes. Third, this trial was conducted at a single medical center in Taiwan. Previous studies have indicated that the clinical presentation and histologic and molecular analyses of the skin in Asian patients with AD differ from those of European/American patients.[50] Another study indicated that the genetic polymorphisms involved in AD in Asian populations differ from those in other ethnic populations.[51] As the pathogenesis of AD may differ from that in Western countries, our results only provide evidence for Asian patients with AD. Fourth, following a 12-week supplementation with LMF, our observations did not reveal any significant alterations in Th2-related cytokine levels, including IL-4, IL-13, and IL-10. To further elucidate the immunomodulatory effects of LMF, it may be worthwhile to explore additional pathways, such as IL-17, IL-22 and IgG. Fifth, we did not administer LMF for an extended period to determine if patients would develop tolerance or resistance with prolonged use. Further research involving the extended use of LMF is warranted.

In conclusion, this is the first randomized, double-blinded, placebo-controlled trial that provides evidence that LMF supplementation could be an effective and safe alternative therapy for patients with AD. LMF was effective not only during the supplementation phase but also during the follow-up phase, indicating better and long-term symptom relief. Supplementation with LMF may decrease the need for topical steroid application. Moreover, AD-related serum immunologic markers, such as serum IgE levels, eosinophil percentage, CD8+ T cell number, IFN-γ levels, ESR, and CRP levels, were significantly decreased in the study group after supplementation with LMF. These results demonstrate that LMF effectively ameliorates AD symptoms via its anti-inflammatory activity. Further research is necessary to comprehensively understand the underlying mechanism behind the immunomodulatory effects of LMF on patients with AD.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Ethical Review

This study was conducted in accordance with the Declaration of Helsinki. The trial protocol was approved by the Institutional Review Board (IRB) of the Chang Gung Medical Foundation (IRB No. 201601520A3C601) and performed in accordance with the recommendations of the guidelines for clinical trials of the same committee.

Informed consent

All participants provided written informed consent.

References

[1] Langan, S. M.; Irvine, A. D.; Weidinger, S. Atopic Dermatitis. *Lancet*. 2020, *396*(10247), 345–360. DOI: 10.1016/S0140-6736(20)31286-1.

[2] Hwang, C. Y.; Chen, Y. J.; Lin, M. W.; Chen, T. J.; Chu, S. Y.; Chen, C. C.; Lee, D. D.; Chang, Y. T.; Wang, W. J.; Liu, H. N. Prevalence of Atopic Dermatitis, Allergic Rhinitis and Asthma in Taiwan: A National Study 2000 to 2007. *Acta Derm. Venereol*. 2010, *90*(6), 589–594. DOI: 10.2340/00015555-0963.

[3] Weidinger, S.; Beck, L. A.; Bieber, T.; Kabashima, K.; Irvine, A. D. Atopic Dermatitis. *Nat. Rev. Dis. Primer*. 2018, *4*(1), 1. DOI: 10.1038/s41572-018-0001-z.

[4] Yang, L.; Fu, J.; Zhou, Y. Research Progress in Atopic March. *Front. Immunol*. 2020, *11*, 1907. DOI: 10.3389/fimmu.2020.01907.

[5] Hsieh, B. J.; Shen, D.; Hsu, C. J.; Chan, T. C.; Cho, Y. T.; Tang, C. H.; Chu, C. Y. The Impact of Atopic Dermatitis on Health-Related Quality of Life in Taiwan. *J. Formosan. Med. Assoc*. 2022, *121*(1), 269–277. DOI: 10.1016/j.jfma.2021.03.024.

[6] Andersen, L.; Nyeland, M. E.; Nyberg, F. Increasing Severity of Atopic Dermatitis is Associated with a Negative Impact on Work Productivity Among Adults with Atopic Dermatitis in France, Germany, the U.K. and the U.S. A. *Br. J. Dermatol*. 2020, *182*(4), 1007–1016. DOI: 10.1111/bjd.18296.

[7] Chan, T. C.; Lin, Y. C.; Cho, Y. T.; Tang, C. H.; Chu, C. Y. Impact of Atopic Dermatitis on Work and Activity Impairment in Taiwan. *Acta Derm. Venereol*. 2021, *101*(9), adv00556. DOI: 10.2340/00015555-3918.

[8] Goh, M. S.; Yun, J. S.; Su, J. C. Management of Atopic Dermatitis: A Narrative Review. *Med. J. Aust*. 2022, *216*(11), 587–593. DOI: 10.5694/mja2.51560.

[9] Koh, L. F.; Ong, R. Y.; Common, J. E. Skin Microbiome of Atopic Dermatitis. *Allergol. Int*. 2022, *71*(1), 31–39. DOI: 10.1016/j.alit.2021.11.001.

[10] Narla, S.; Silverberg, J. I. Dermatology for the Internist: Optimal Diagnosis and Management of Atopic Dermatitis. *Ann. Med*. 2021, *53*(1), 2165–2177. DOI: 10.1080/07853890.2021.2004322.

[11] Yang, N.; Chen, Z.; Zhang, X.; Shi, Y. Novel Targeted Biological Agents for the Treatment of Atopic Dermatitis. *BioDrug*. 2021, *35*(4), 401–415. DOI: 10.1007/s40259-021-00490-x.

[12] Matterne, U.; Bohmer, M. M.; Weisshaar, E.; Jupiter, A.; Carter, B.; Apfelbacher, C. J. Oral H1 Antihistamines as 'Add-on' Therapy to Topical Treatment for Eczema. *Cochrane Database Syst. Rev*. 2019, *2019*(1), CD012167. DOI: 10.1002/14651858.CD012167.pub2.

[13] Li, A. W.; Yin, E. S.; Antaya, R. J. Topical Corticosteroid Phobia in Atopic Dermatitis: A Systematic Review. *JAMA Dermato*. 2017, *153*(10), 1036–1042. DOI: 10.1001/jamadermatol.2017.2437.

[14] Bos, B.; Antonescu, I.; Osinga, H.; Veenje, S.; de Jong, K.; de Vries, T. W. Corticosteroid Phobia (Corticophobia) in Parents of Young Children with Atopic Dermatitis and Their Health Care Providers. *Pediatr. Dermatol*. 2019, *36*(1), 100–104. DOI: 10.1111/pde.13698.

[15] Agache, I.; Song, Y.; Posso, M.; Alonso-Coello, P.; Rocha, C.; Sola, I.; Beltran, J.; Akdis, C. A.; Akdis, M.; Brockow, K., et al. Efficacy and Safety of Dupilumab for Moderate-To-Severe Atopic Dermatitis: A Systematic Review for the Eaaci Biologicals Guidelines. *Allergy (Oxford, U. K.)*. 2021, *76*(1), 45–58. DOI: 10.1111/all.14510.

[16] Lee, E. M.; Cho, Y. T.; Hsieh, W. T.; Chan, T. C.; Shen, D.; Chu, C. Y.; Tang, C. H. Healthcare Utilization and Costs of Atopic Dermatitis in Taiwan. *J. Formosan. Med. Assoc*. 2022, *121*(10), 1963–1971. DOI: 10.1016/j.jfma.2022.01.028.

[17] Ye, J.; Li, Y.; Teruya, K.; Katakura, Y.; Ichikawa, A.; Eto, H.; Hosoi, M.; Hosoi, M.; Nishimoto, S.; Shirahata, S. Enzyme-Digested Fucoidan Extracts Derived from Seaweed Mozuku of Cladosiphon Novae-Caledoniae Kylin Inhibit Invasion and Angiogenesis of Tumor Cells. *Cytotechnology*. 2005, *47*(1–3), 117–126. DOI: 10.1007/s10616-005-3761-8.

[18] Chen, M. C.; Hsu, W. L.; Hwang, P. A.; Chou, T. C. Low Molecular Weight Fucoidan Inhibits Tumor Angiogenesis Through Downregulation of Hif-1/vegf Signaling Under Hypoxia. *Mar. Drugs*. 2015, *13*(7), 4436–4451. DOI: 10.3390/md13074436.

[19] Apostolova, E.; Lukova, P.; Baldzhieva, A.; Katsarov, P.; Nikolova, M.; Iliev, I.; Peychev, L.; Trica, B.; Oancea, F.; Delattre, C., et al. Immunomodulatory and Anti-Inflammatory Effects of Fucoidan: A Review. *Polymer (Basel)*. 2020, *12*(10). DOI: 10.3390/polym12102338.

[20] Chen, B. R.; Li, W. M.; Li, T. L.; Chan, Y. L.; Wu, C. J. Fucoidan from Sargassum Hemiphyllum Inhibits Infection and Inflammation of Helicobacter Pylori. *Sci. Rep*. 2022, *12*(1), 429. DOI: 10.1038/s41598-021-04151-5.

[21] Iwamoto, K.; Hiragun, T.; Takahagi, S.; Yanase, Y.; Morioke, S.; Mihara, S.; Kameyoshi, Y.; Hide, M. Fucoidan Suppresses Ige Production in Peripheral Blood Mononuclear Cells from Patients with Atopic Dermatitis. *Arch. Dermatol. Res*. 2011, *303*(6), 425–431. DOI: 10.1007/s00403-010-1115-7.

[22] Chen, B. R.; Hsu, K. T.; Li, T. L.; Chan, Y. L.; Wu, C. J. Topical Application of Fucoidan Derived from Cladosiphon Okamuranus Alleviates Atopic Dermatitis Symptoms Through Immunomodulation. *Int. Immunopharmacol.* 2021, *101*(Pt B), 108362. DOI: 10.1016/j.intimp.2021.108362.
[23] Chen, B. R.; Hsu, K. T.; Hsu, W. H.; Lee, B. H.; Li, T. L.; Chan, Y. L.; Wu, C. J. Immunomodulation and Mechanisms of Fucoidan from Cladosiphon Okamuranus Ameliorates Atopic Dermatitis Symptoms. *Int. J. Biol. Macromol.* 2021, *189*, 537–543. DOI: 10.1016/j.ijbiomac.2021.08.001.
[24] Hwang, P. A.; Phan, N. N.; Lu, W. J.; Ngoc Hieu, B. T.; Lin, Y. C. Low-Molecular-Weight Fucoidan and High-Stability Fucoxanthin from Brown Seaweed Exert Prebiotics and Anti-Inflammatory Activities in Caco-2 Cells. *Food. Nutr. Res.* 2016, *60*(1), 32033. DOI: 10.3402/fnr.v60.32033.
[25] Yan, M. D.; Lin, H. Y.; Hwang, P. A. The Anti-Tumor Activity of Brown Seaweed Oligo-Fucoidan via Lncrna Expression Modulation in Hepg2 Cells. *Cytotechnology.* 2019, *71*(1), 363–374. DOI: 10.1007/s10616-019-00293-7.
[26] Kunz, B.; Oranje, AP.; Labreze, L.; Stalder, JF.; Ring, J.; Taieb, A. Severity Scoring of Atopic Dermatitis: The Scorad Index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatol. (Basel, Switz.)* 1993, *186* (1), 23–31. DOI: 10.1159/000247298.
[27] Kunz, B.; Oranje, A. P.; Labreze, L.; Stalder, J. F.; Ring, J.; Taieb, A. Clinical Validation and Guidelines for the Scorad Index: Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatol. (Basel, Switz.).* 1997, *195*(1), 10–19. DOI: 10.1159/000245677.
[28] Broeders, J. A.; Ahmed Ali, U.; Fischer, G. Systematic Review and Meta-Analysis of Randomized Clinical Trials (Rcts) Comparing Topical Calcineurin Inhibitors with Topical Corticosteroids for Atopic Dermatitis: A 15-Year Experience. *J. Am. Acad. Dermatol.* 2016, *75*(2), 410–419 e413. DOI: 10.1016/j.jaad.2016.02.1228.
[29] He, A.; Feldman, S. R.; Fleischer, A. B., Jr. An Assessment of the Use of Antihistamines in the Management of Atopic Dermatitis. *J. Am. Acad. Dermatol.* 2018, *79*(1), 92–96. DOI: 10.1016/j.jaad.2017.12.077.
[30] Tomori, M.; Nagamine, T.; Miyamoto, T.; Iha, M. Evaluation of the Immunomodulatory Effects of Fucoidan Derived from Cladosiphon Okamuranus Tokida in Mice. *Mar. Drugs.* 2019, *17*(10). DOI: 10.3390/md17100547.
[31] Bieber, T. Atopic Dermatitis. *N. Engl. J. Med.* 2008, *358*(14), 1483–1494. DOI: 10.1056/NEJMra074081.
[32] Makowska, K.; Nowaczyk, J.; Blicharz, L.; Waskiel-Burnat, A.; Czuwara, J.; Olszewska, M.; Rudnicka, L. Immunopathogenesis of Atopic Dermatitis: Focus on Interleukins as Disease Drivers and Therapeutic Targets for Novel Treatments. *Int. J. Mol. Sci.* 2023, *24*(1). DOI: 10.3390/ijms24010781.
[33] Trier, A. M.; Kim, B. S. Insights into Atopic Dermatitis Pathogenesis Lead to Newly Approved Systemic Therapies. *Br. J. Dermatol.* 2023, *188*(6), 698–708. DOI: 10.1093/bjd/ljac016.
[34] Munera-Campos, M.; Carrascosa, J. M.; Innovation in Atopic Dermatitis: From Pathogenesis to Treatment. *Actas Dermo-Sifiliogr. (Engl. Ed.)* 2020, *111*(3), 205–221. doi 10.1016/j.adengl.2020.03.001.
[35] Mack, M. R.; Kim, B. S. The Itch-Scratch Cycle: A Neuroimmune Perspective. *Trend. Immunol.* 2018, *39*(12), 980–991. DOI: 10.1016/j.it.2018.10.001.
[36] Gandhi, N. A.; Bennett, B. L.; Graham, N. M.; Pirozzi, G.; Stahl, N.; Yancopoulos, G. D. Targeting Key Proximal Drivers of Type 2 Inflammation in Disease. *Nat. Rev. Drug. Discov.* 2016, *15*(1), 35–50. DOI: 10.1038/nrd4624.
[37] Criado, P. R.; Miot, H. A.; Ianhez, M. Eosinophilia and Elevated Ige Serum Levels: A Red Flag: When Your Diagnosis is Not a Common Atopic Eczema or Common Allergy. *Inflamm. Res.* 2023, *72*(3), 541–551. DOI: 10.1007/s00011-023-01690-7.
[38] Fagundes, B. O.; de-Sousa, T. R.; Victor, J. R. Gamma-Delta (Gammadelta) T Cell-Derived Cytokines (Il-4, Il-17, Ifn-Gamma and Il-10) and Their Possible Implications for Atopic Dermatitis Development. *Int. J. Dermatol.* 2023, *62*(4), 443–448. DOI: 10.1111/ijd.16364.
[39] Seneviratne, S. L.; Jones, L.; Bailey, A. S.; Black, A. P.; Ogg, G. S. Severe Atopic Dermatitis is Associated with a Reduced Frequency of Il-10 Producing Allergen-Specific Cd4+ T Cells. *Clin. Exp. Dermatol.* 2006, *31*(5), 689–694. DOI: 10.1111/j.1365-2230.2006.02172.x.
[40] Kaesler, S.; Volz, T.; Skabytska, Y.; Koberle, M.; Hein, U.; Chen, K. M.; Guenova, E.; Wolbing, F.; Rocken, M.; Biedermann, T. Toll-Like Receptor 2 Ligands Promote Chronic Atopic Dermatitis Through Il-4-Mediated Suppression of Il-10. *J. Allergy. Clin. Immunol.* 2014, *134*(1), 92–99. DOI: 10.1016/j.jaci.2014.02.017.
[41] Brunner, P. M.; Suarez-Farinas, M.; He, H.; Malik, K.; Wen, H. C.; Gonzalez, J.; Chan, T. C.; Estrada, Y.; Zheng, X.; Khattri, S., et al. The Atopic Dermatitis Blood Signature is Characterized by Increases in Inflammatory and Cardiovascular Risk Proteins. *Sci. Rep.* 2017, *7*(1), 8707.
[42] Guttman-Yassky, E.; Ungar, B.; Malik, K.; Dickstein, D.; Suprun, M.; Estrada, Y. D.; Xu, H.; Peng, X.; Oliva, M.; Todd, D., et al. Molecular Signatures Order the Potency of Topically Applied Anti-Inflammatory Drugs in Patients with Atopic Dermatitis. *J. Allergy Clin. Immunol.* 2017, *140*(4), 1032–1042 e1013.
[43] Zhao, J.; Chen, Z. Y.; Li, L. F. Association Between the Il-10-1082g/a, Il-10-592a/C, and Il-10-819g/a Polymorphisms and Atopic Dermatitis Susceptibility: A Meta-Analysis. *Genet. Test. Mol. Biomarker.* 2019, *23* (5), 332–341. DOI: 10.1089/gtmb.2018.0276.
[44] Bieber, T. Atopic Dermatitis. *Ann. Dermatol.* 2010, *22*(2), 125–137. DOI: 10.5021/ad.2010.22.2.125.
[45] Tawada, C.; Kanoh, H.; Nakamura, M.; Mizutani, Y.; Fujisawa, T.; Banno, Y.; Seishima, M. Interferon-Gamma Decreases Ceramides with Long-Chain Fatty Acids: Possible Involvement in Atopic Dermatitis and Psoriasis. *J. Invest. Dermatol.* 2014, *134*(3), 712–718. DOI: 10.1038/jid.2013.364.

[46] Hijnen, D.; Knol, E. F.; Gent, Y. Y.; Giovannone, B.; Beijn, S. J.; Kupper, T. S.; Bruijnzeel-Koomen, C. A.; Clark, R. A. Cd8(+) T Cells in the Lesional Skin of Atopic Dermatitis and Psoriasis Patients are an Important Source of Ifn-Gamma, Il-13, Il-17, and Il-22. *J. Invest. Dermatol.* 2013, *133*(4), 973–979. DOI: 10.1038/jid.2012.456.

[47] Nograles, K. E.; Zaba, L. C.; Shemer, A.; Fuentes-Duculan, J.; Cardinale, I.; Kikuchi, T.; Ramon, M.; Bergman, R.; Krueger, J. G.; Guttman-Yassky, E. Il-22-Producing "T22" T Cells Account for Upregulated Il-22 in Atopic Dermatitis Despite Reduced Il-17-Producing Th17 T Cells. *J. Allergy Clin. Immunol.* 2009, *123*(6), 1244–1252 e1242. DOI: 10.1016/j.jaci.2009.03.041.

[48] Sgnotto, F. D. R.; de Oliveira, M. G.; Lira, A. A. L.; Inoue, A. H. S.; Titz, T. O.; Orfali, R. L.; Bento-de-Souza, L.; Sato, M. N.; Aoki, V.; Duarte, A. J. S., et al. Igg from Atopic Dermatitis Patients Induces Il-17 and Il-10 Production in Infant Intrathymic Tcd4 and Tcd8 Cells. *Int. J. Dermatol.* 2018, *57*(4), 434–440. DOI: 10.1111/ijd.13907.

[49] Santos, L. S.; Sgnotto, F. D. R.; Sousa, T. R.; Orfali, R. L.; Aoki, V.; Duarte, A.; Victor, J. R. Igg from Atopic Dermatitis Patients Induces Non-Atopic Infant Thymic Invariant Natural Killer T (Inkt) Cells to Produce Il-4, Il-17, and Il-10. *Int. J. Dermatol.* 2020, *59*(3), 359–364. DOI: 10.1111/ijd.14688.

[50] Noda, S.; Suarez-Farinas, M.; Ungar, B.; Kim, S. J.; de Guzman Strong, C.; Xu, H.; Peng, X.; Estrada, Y. D.; Nakajima, S.; Honda, T., et al. The Asian Atopic Dermatitis Phenotype Combines Features of Atopic Dermatitis and Psoriasis with Increased Th17 Polarization. *J. Allergy Clin. Immunol.* 2015, *136*(5), 1254–1264.

[51] Cheng, J.; Wu, J. J.; Han, G. Epidemiology and Characterization of Atopic Dermatitis in East Asian Populations: A Systematic Review. *Dermatol. Ther. (Heidelb.).* 2021, *11*(3), 707–717. DOI: 10.1007/s13555-021-00516-w.