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Therapeutic efficacy and mechanism of solubilized sturgeon oil in a mouse model of house dust mite-induced atopic dermatitis

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ABSTRACT

Fish oil supplementation exerts therapeutic effects on atopic dermatitis (AD). The therapeutic effects and mechanisms of action of solubilized sturgeon oil (SSO) prepared from sturgeon oil in AD were examined. NC/Nga mice were topically treated with house dust mite (HDM) extract to induce AD and were then treated with SSO, which exerted potent therapeutic effects on AD symptoms. SSO reduced epidermal hyperplasia and suppressed mast cell infiltration in the dorsal skin. It significantly reduced total and HDM-specific serum IgE levels in AD mice. SSO significantly downregulated interleukin (IL)-4, IL-5, IL-13, IL-17A, and cyclooxygenase-2, while upregulating IL-10 and tight junction-related proteins filaggrin, claudin-1, occludin, and ZO-1 in AD skin tissues. These results show that SSO exerts therapeutic effects in AD mice by alleviating lesions, downregulating IgE, inflammatory mediators, and Th2 cytokines, and upregulating IL-10 and tight junction-related proteins. Thus, SSO may be a promising natural product for AD treatment.

1. Introduction

Atopic dermatitis (AD) is a complex inflammatory skin disorder characterized by chronic, relapsing, pruritic, and inflammatory eczematous eruptions that usually develop during early childhood (Bieber et al., 2010; Weidinger & Novak, 2016). The prevalence and incidence of AD have increased over the past several decades, affecting 15–20 % of the children and 10 % of the adults (Nutten, 2015; Hadi et al., 2021). AD has a complex etiology involving genetic and environmental factors, skin barrier dysfunction, and immune dysregulation (Avena-Woods, 2017; Yang et al., 2020; Song et al., 2022). Immune dysfunction, which involves increased levels of serum immunoglobulin E (IgE) and production of type 2 helper T cell (Th2)-specific cytokines, has been studied as a major mechanism of AD (David et al., 2017; Kapur et al., 2018). Most patients with AD exhibit hyperproduction of IgE, particularly during disease onset or flares. IgE-dependent late-phase reactions, which involve the activation and degranulation of mast cells, contribute to chronic inflammatory responses in AD (Kawakami et al., 2009). The predominance of Th2 cytokines such as interleukin (IL)-4 and IL-13 in skin lesions is a notable feature of AD. The key role of Th2 cytokines in AD pathology has been proven in a clinical study, which has shown that dupilumab, a monoclonal antibody (mAb) that blocks the IL-4/IL-13 receptor, is effective in AD treatment (Roesner et al., 2016).

Treatments for AD include topical agents such as moisturizers, corticosteroids, calcineurin inhibitors, antibiotics, phototherapy, and systemic medications such as corticosteroids, cyclosporine, methotrexate, mycophenolate mofetil, and azathioprine (Eichenfield et al., 2014; Ghamrawi et al., 2020; Li et al., 2021; Calabrese et al., 2022). However, most current mainstay treatments have limited efficacy and potentially serious side effects, especially in children (Coondoo et al., 2014). Topical corticosteroids, which suppress antigen processing and proinflammatory cytokine release in immune cells, have been used as first-line treatments for AD (Andersen et al., 2017). However, long-term use of steroids poses risks of skin atopy, muscle weakness, facial

Abbreviations: AD, atopic dermatitis; ALA, α-linolenic acid; COX-2, cyclooxygenase-2; DHA, docosahexaenoic acids; ELISA, enzyme-linked immunosorbent assay; HDM, house dust mite; H&E, hematoxylin and eosin; IFN-γ, interferon-γ; IgE, immunoglobulin E; IL, interleukin; LA, linoleic acid; mAb, monoclonal antibody; GLA, γ-linolenic acid; PUFA, polyunsaturated fatty acid; qRT-PCR, quantitative real-time polymerase chain reaction; SSO, solubilized sturgeon oil; Th2, type 2 helper T cell.

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edema, psoriasis, and bleeding, as well as nonspecific suppression of immune responses (Walling & Swick, 2010; Hajar et al., 2015). Therefore, the development of alternative therapeutics for AD treatment remains a global challenge.

Natural products containing rich amounts of polyphenols and flavonoids with anti-inflammatory activity have been explored for the treatment of AD. Numerous studies have shown that patients may benefit from herbal medicines (Vieira et al., 2016; Tumpang et al., 2018; Kwon et al., 2020; Hon et al., 2022). Fish oil containing long-chain polyunsaturated fatty acids (PUFAs) has also been shown to have beneficial effects on AD management (Sardana & Sachdeva, 2022). In clinical studies, linoleic acid (LA)-containing water-in-oil emulsions have been demonstrated to improve skin barrier dysfunction and the clinical severity of AD (Nasrollahi et al., 2018). γ-linolenic acid (GLA) was also effective and had no side-effects in the treatment patients with AD (Fiocchi et al., 1994; Andreassi et al., 1997; Simon et al., 2014). Sturgeon oil, which is rich in PUFAs, is known to be effective in treating skin burns (Chen et al., 2022). Sturgeon oil has been used as a traditional medicine to cure skin conditions in certain Eastern European countries. However, the effect of sturgeon oil on AD has not yet been investigated.

Here, we report solubilized sturgeon oil (SSO) as a novel therapeutic natural product for AD. SSO is an oil-in-water formulation of sturgeon oil, prepared via nanoemulsification and distillation. The advantage of making sturgeon oil into an oil-in-water microemulsion is that the skin absorption rate of the oil contained in the microemulsion is higher than the absorption rate of the oil itself, and the microemulsion is easy to formulate into a gel-type ointment for topical application. In addition, the emulsion can be added directly to cell culture media, but the oil itself cannot be used to investigate its effects in cell culture systems. The therapeutic effects of SSO were examined in NC/Nga mice, a well-established animal model of AD. Our results showed that SSO was effective in treating house dust mite (HDM)-induced AD-like lesions in NC/Nga mice.

2. Materials and methods

2.1. Preparation and analysis of SSO

The methods for SSO preparation are described in detail in a patent registered with the Korean Intellectual Property Office (Lee, 2023). Briefly, the oil layer was separated from a boiling-water extract of the entire body of a Siberian sturgeon (*Acipenser baerii*). Sturgeon oil was mixed with purified water (1:100) and then sprayed repeatedly onto a stainless steel wall using a high pressure syringe pump to generate a nanoemulsion with a very small droplet size. The nanoemulsion was mixed with an herbal extract (200:1) prepared using wild ginseng and other medicinal plants, and the mixture was aged in a sterile container for at least 10 d at 19 ~ 23 °C. The aged mixture was then distilled in a stainless-steel water distiller to obtain SSO. The total lipid content and fatty acid composition were analyzed by the Korea Quality Testing Institute (Suwon, Korea). The total lipid content of SSO was 0.53 ± 0.06 %, as determined by the Soxhlet-petroleum ether extraction method. The fatty acid composition was analyzed using gas chromatography-mass spectrometry after derivatization to fatty acid methyl esters (Chiu & Kuo, 2020), and the results are shown in Table 1. SSO was used as such for oral administration, or formulated into a semisolid gel by adding carbomer (Lubrizol Korea Co., Seoul, Korea) and L-arginine (Saesang Co., Seoul, Korea) at a ratio of 1:1 to a final concentration of 1.0 % at 50 °C for topical application to AD skins.

2.2. Animals

Ten-week-old male NC/Nga mice were purchased from Central Laboratory Animal Inc. (Seoul, Korea) and housed in an air-conditioned room at 24 ± 2℃ with 55 ± 15 % humidity, under a 12 h light–dark cycle. The mice were individually placed in separate cages. Female C57BL/6 mice (8–12 weeks old) were purchased from Kosa Bio, Inc. (Seongnam, Korea). All animal experimental procedures were performed in accordance with the guidelines and regulations approved by the Institutional Animal Care and Use Committee of Chungbuk National University (CBNUA-1491-21-02).

Table 1
The fatty acid composition of SSO.

Fatty acid	Quantity (μg/ml)		
Lauric acid	4.3	±	0.2
Tridecanoic acid	3.7	±	0.1
Myristic acid	4.5	±	0.2
Myristoleic acid	864.4	±	23.4
cis-10-Pentadecenoic acid	77.8	±	3.66
Palmitic acid	684.0	±	34.2
Palmitoleic acid	1,389.2	±	85.6
Heptadecanoic acid	32.2	±	1.4
cis-10-Heptadecenoic	27.6	±	1.2
Stearic acid	12.3	±	1.1
cis-Oleic acid	552.6	±	26.7
trans-Linoleic acid	1,867,8	±	96.4
α-Linolenic acid	241.0	±	9.8
γ-Linolenic acid	76.0	±	2.8
Arachidoic acid	23.2	±	1.2
cis-11-Eicosenoic acid	41.9	±	1.1
cis-11,14-Eicosadienoic acid	4.8	±	0.6
cis-11,14,17-Eicosadienoic acid	31.1	±	1.2
cis-8,11,14-Eicosadienoic acid	71.0	±	2.8
Behenic acid	50.4	±	9.2
Erucic acid	4.24	±	0.4
cis-13,16-Docosadienoic acid	3.33	±	0.3
cis-4,7,10,13,16,19-Docosahexanoic acid	7.60	±	1.1
Lignoceric acid	2.90	±	0.2

2.3. Induction and treatment of AD skin lesions

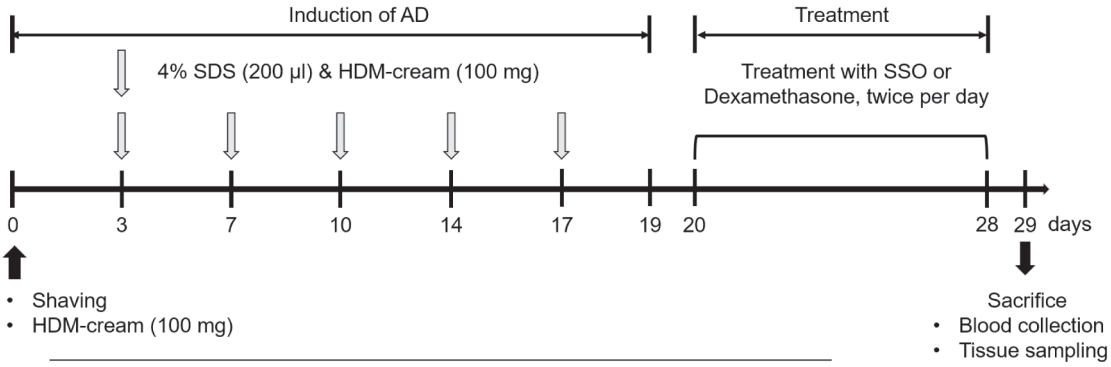
Hair on the upper back of male NC/Nga mice (11 weeks old) was shaved with a clipper and shaver. Skin barrier function was disrupted using 200 μL/mouse of 4 % (w/v) sodium dodecyl sulfate treatment 30 min before application of the HDM cream (Biostir, Kobe, Japan), a hydrophilic petrolatum-based ointment containing extract of HDM *Dermatophagoides farinae*, to the shaved dorsal and ear skin, as described previously (Yamamoto et al., 2007; Matsuda et al., 1997). HDM cream (100 mg/mouse) was treated twice per week for 3 weeks. After induction of AD, the mice were divided randomly into five groups of five mice each and treated with SSO or dexamethasone (0.1 %) ointment. AD induction treatment regimens are shown in Fig. 1. In groups treated with SSO, SSO was administered orally at 100 μL/mouse or topically at 100 μL/mouse, a dose sufficient to cover AD-like skin lesions, twice per day for 9 consecutive days. Mice were euthanized on day 29, and the dorsal skin and blood were collected for analysis. Serum samples were stored at −80℃ until further use.

2.4. Scoring of AD symptoms

AD severity was evaluated according to the degree of five symptoms, namely erythema, edema, erosion, dryness, and lichenification, which were individually scored as 0 (no symptoms), 1 (mild), 2 (moderate), or 3 (severe) (Hwang et al., 2019). The sum of the individual scores was used as the dermatitis severity score. The dermatitis severity scored ranges from 0 to 15.

2.5. Measurement of total IgE and HDM-specific IgE levels in serum

Serum levels of total IgE were measured using an enzyme-linked immunosorbent assay (ELISA) kit (BD Biosciences, San Jose, CA, USA), according to manufacturer's instructions. HDM-specific IgE levels were measured using direct ELISA. Briefly, a 96-well immunoplate was coated with 100 μg/mL HDM extract at 4℃ overnight. The plates were



Group name	HDM-cream	Treatment regimen
Neg	-	-
Pos	+	-
Dexa	+	Dexamethasone ointment
Feed	+	Oral feeding with SSO
Topical	+	Topical application with SSO
Fd+Tp	+	Oral feeding and topical application

Fig. 1. Experimental design for AD induction and SSO treatment in NC/Nga mice. (A) Schematic diagram for induction of AD with HDM cream and treatment with SSO. (B) Detailed information for the groups. Neg, AD-negative group; Pos, AD-positive group whose AD skins were treated with distilled water-containing gel; Dexa, mice treated with 0.1 % dexamethasone ointment; Feed, mice orally administered SSO; Topical, mice treated topically with SSO-containing gel; Fd + Tp, mice orally administered SSO (Fd) and treated topically (Tp) with SSO. AD, atopic dermatitis; SSO, solubilized sturgeon oil; HDM, house dust mite.

then washed three times with phosphate-buffered saline containing 0.1 % Tween-20 and blocked by incubation with 3 % bovine serum albumin for 1 h at room temperature. Serum samples were added to the plates and incubated for 2 h at room temperature, followed by treatment with biotin-labeled rat anti-mouse IgE (BD Pharmingen, CA, USA). The plates were washed three times and incubated with streptavidin-labeled horse radish peroxidase (BD Pharmingen) for 30 min at room temperature. The enzymatic reactions were developed using the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (BD Pharmingen) and terminated by adding 2 mM H_2SO_4. The absorbance of the plate was measured at 450 nm using a microplate reader.

2.6. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from mouse skin or cultured Th2 cells using a Hybrid-R RNA purification kit (GeneAll, Seoul, Korea) and reverse transcribed to cDNA using the ReverTra Ace qPCR RT Master Mix (Takara, Tokyo, Japan). The levels of cytokine mRNAs relative to β-actin were measured via qRT-PCR on a CFX Connect Real-Time System (Bio-Rad Laboratories, CA, USA) using TB Green® Premix Ex Taq™ II (Takara). The primer sequences are shown in Table 2.

2.7. Histological analyses

The dorsal skin of each mouse was fixed in 10 % formalin for 24 h. The skin specimens were then embedded in paraffin and cut into 5 μm sections. The skin sections were stained with hematoxylin and eosin (H&E) or toluidine blue to examine epidermal thickness and degree of mast cell infiltration, respectively. The H&E- and toluidine blue-stained skin specimens were observed under a microscope (Leica DMi8, Leica, Wetzlar, Germany). Mast cells were counted at three randomly designated sites using a microscope.

2.8. Th2 cell differentiation and stimulation

Naive $CD4^+$ T cells in the spleen of C57BL/6 mice were isolated using a naive $CD4^+$ T cell isolation kit (Miltenyi Biotec, CA, USA). The naive $CD4^+$ T cells were seeded into 24-well plates coated with anti-CD3 mAb (5 μg/mL) and anti-CD28 mAb (2 μg/mL). For Th2 polarization, naive $CD4^+$ T cells were stimulated with IL-2 (30 unit/mL), IL-4 (100 ng/mL), and anti-interferon-gamma (IFN-γ) mAb (5 μg/mL). The cells were harvested 5 d later and re-stimulated in a 96-well plate coated with anti-CD3 mAb (5 μg/mL) and anti-CD28 mAb (2 μg/mL) for 2 d in the presence of varying concentrations of SSO. The culture supernatants were collected, and cytokines were measured using the BD OptEIA ELISA Set kit (BD Biosciences) for mouse IL-4 and IL-5 or the DuoSet ELISA kit (R&D Systems, MN, USA) for mouse IL-10 and IL-13, according to the manufacturer's instructions.

Table 2
Sequences of primers used for qRT-PCR.

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
IL-4	TGGTGGGAGACTTACCTGATG	CCCGGATAACTTCACAACTTCTT
IL-5	ATGACTGTGCCTCTGTGCCTGGAGC	CGGGAAGACAATAACTGCACCC
IL-10	CGGGAAGACAATAACTGCACCC	CGGTTAGCAGTATGTTGTCCAGC
IL-13	CACACAAGACCAGACTCCCC	TCTGGGTCCTGTAGATGGCA
IL-17A	TGAAAACACAGAAGTAACGTCCG	CCCAGGAGGAAATTGTAATGGGA
COX-2	CGTGGTCACTTTACTACGAG	AGGTACATAGTAGTCCTGAGC
Filaggrin	CTAGAGGGCATGAGTGTAGTCA	CAAGACTGGACAGTTGGCTGG
β-actin	AGAGGGAAATCGTGCGTGAC	CAATAGTGATGACCTGGCCGT

2.9. Western blot

Total protein was isolated from dorsal skin tissue as previously described (Im et al., 2016). Protein concentration was measured using the Bicinchoninic Acid Protein Assay kit (Pierce, IL, USA). Equal amounts of proteins were separated by acrylamide gel electrophoresis and transferred to PVDF membranes (Merck, Germany), which were then blocked with Tris-buffered saline containing Tween 20 (TBST) supplemented with 5 % bovine serum albumin. The primary antibodies for filaggrin (Santa Cruz Biotechnology, Texas, USA), claudin-1 (Santa Cruz), occludin (Cell Signaling Technology, MA, USA), ZO-1 (Santa Cruz), and β-Actin (Cell Signaling Technology) were probed onto the PVDF membrane overnight at 4 °C. All the primary antibodies were diluted 1:1,000 using 5 % BSA in TBS-T solution. Then, the secondary antibody, which was diluted at 1:2,000 in the TBS-T solution, was incubated for 10 min at room temperature. To detect the target protein, the chemiluminescent substrate was reacted with the horseradish peroxidase conjugated with the secondary antibody for 1 min at room temperature. Target protein bands were observed and captured by Viber Fusion Solo S (Vilber Lourmat, France), and were quantified and normalized using Evolution-capt Edge software (Vilber Lourmat).

2.10. Statistical analysis

The results were analyzed using GraphPad Prism 5 software (GraphPad Software Inc., CA, USA). Data are expressed as the mean ± standard deviation (S.D.). Student's *t*-test was used to compare individual treatments with the control. Statistical significance was set at *P*-value < 0.05.

3. Results

3.1. SSO ameliorates HDM-induced AD-like symptoms in NC/Nga mice

To evaluate the therapeutic effects of SSO on AD, HDM-containing cream was applied to the dorsal and ear skin of NC/Nga mice twice a week for 3 weeks. Repeated exposure to the HDM cream induced AD-like symptoms in mice, including severe scarring, erythema, hemorrhage, excoriation, erosion, and lichenification. The mice with fulminant AD-like symptoms were randomly divided into five groups. At this time point, the average of the sum of severity score of each group was 14.0 ± 0.6. The mice were treated with SSO or dexamethasone ointment. Treatment with SSO significantly reduced the AD-like symptoms (Fig. 2A). In particular, topical application of SSO had a more prominent therapeutic effect than oral administration of SSO, which exerted a therapeutic effect almost comparable to that of the topical application of dexamethasone in terms of histological appearance (Fig. 2A) and severity scoring (Fig. 2C). At the end of the experiments (Day 29), t severity score of the SSO-topical application group was 5.9 ± 0.6, while that of the untreated positive group was 14.3 ± 0.5. The combination therapy with oral administration and topical application of SSO had the most potent therapeutic activity, with a final severity score of 4.6 ± 0.9. Changes in the body weight also supported the therapeutic effects of SSO on AD. The average body weight of the AD-induced group gradually decreased as the AD-symptoms became severe. However, the body weight recovered gradually, reaching the body weight of normal mice after 9 d of SSO treatment (Fig. 2B). Notably, the body weight of mice in the dexamethasone-treated group did not recover, even though dexamethasone exerted therapeutic effects on AD, and this is most likely due to its toxic side effects. These results indicate that SSO ameliorated HDM-induced AD-like symptoms in NC/Nga mice.

3.2. SSO reduces serum levels of total IgE and HDM-specific IgE

Total serum IgE levels were significantly higher in the AD-positive group than the AD-negative group (8364.98 ± 609.61 ng/mL vs. 459.75 ± 12.83 ng/mL, respectively; Fig. 3A). Treatment with SSO or dexamethasone reduced the serum levels of total IgE. The percentages of reduction in total serum IgE levels was 62.2 %, 59.1 %, and 77.3 % in the SSO-feed, SSO-topical application, and SSO-feed/topical application

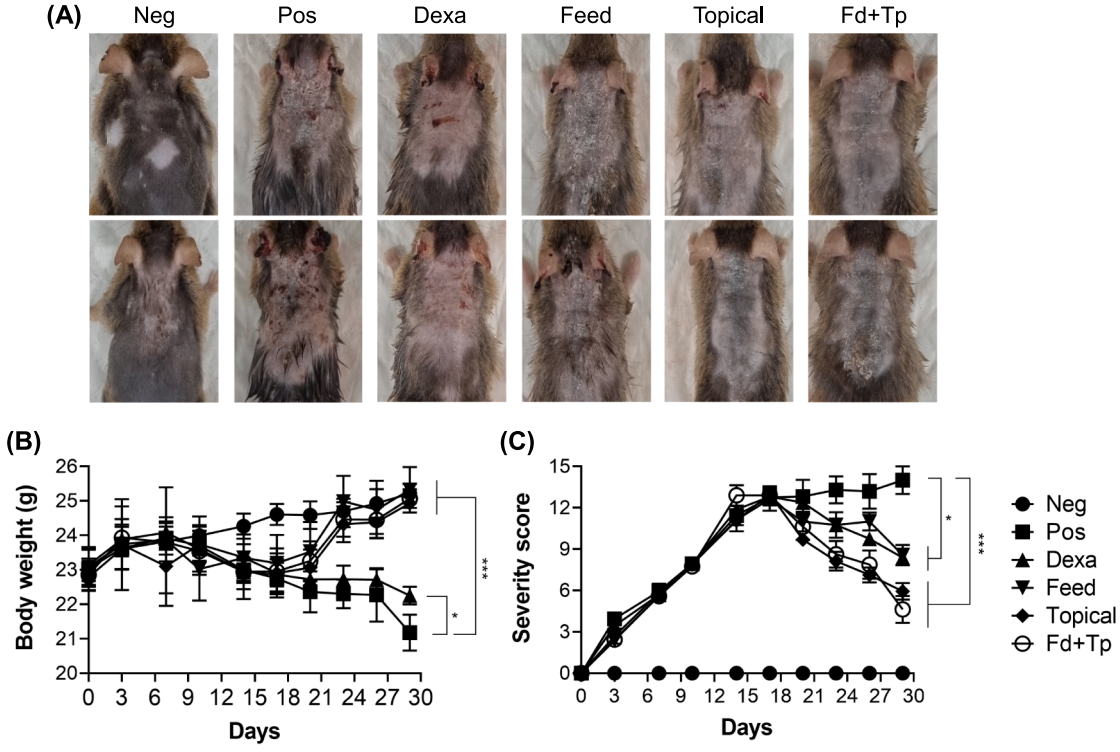


Fig. 2. Therapeutic effect of SSO on the clinical features of AD symptoms. (A) Photographs showing clinical features of AD skins taken at the end of the experiment. (B) Body weight changes. (C) Changes in skin severity scores. The number of mice in each group was 5. * $p < 0.05$, *** $p < 0.001$.

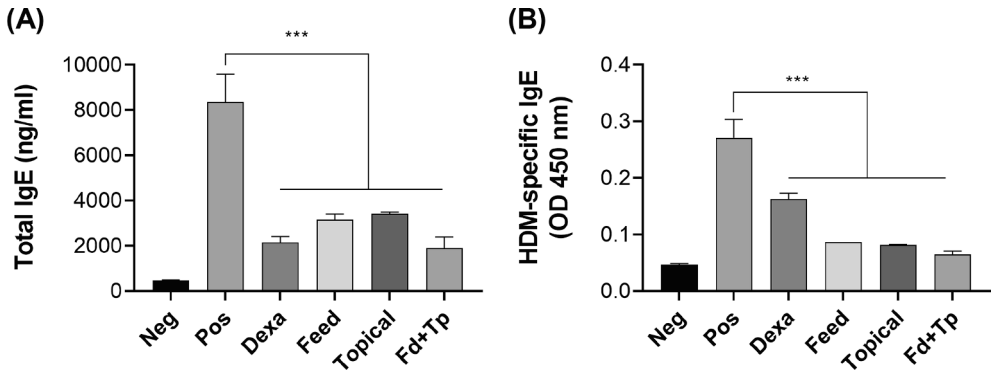


Fig. 3. Effects of SSO on serum IgE concentration. (A) Serum levels of total IgE. (B) Serum levels of HDM-specific IgE. The levels of serum IgE were measured at the end of the experiment. The data are shown as the mean ± S.D. *** $p < 0.001$.

groups, respectively. Similarly, the serum levels of HDM-specific IgE were also significantly reduced by SSO treatment (Fig. 3B). Compared to that in AD-positive group, the serum level of HDM-specific IgE decreased by 67.8 %, 69.9 %, and 76.1 % in the SSO-feed, SSO-topical application, and SSO-feed/topical application groups, respectively. These results show that SSO reduced both the total IgE and HDM-specific IgE levels in the serum.

3.3. SSO decreases epidermis thickening and infiltration of mast cells

HDM-induced AD-like skin lesions have typical microscopic characteristics, including damaged skin tissues, increased epidermal thickness, and mast cell infiltration. SSO treatment significantly attenuated these symptoms. H&E stained skin sections showed that SSO treatment decreased epidermal and dermal thickness and mast cell infiltration (Fig. 4A). Epidermal thickness increased from 14.1 ± 0.4 μm in the AD-negative group to 118.8 ± 7.9 μm in the AD-positive group. This increased epidermal thickness decreased to 72.9 ± 3.5, 53.1 ± 4.9, and 27.1 ± 1.7 μm in the SSO-feed, SSO-topical application, and SSO-feed/topical application groups, respectively (Fig. 4A and 4C). The number of infiltrated mast cells increased from 11.0 ± 0.5 in the AD-negative group to 226.3 ± 8.3 in the AD-positive group. The number of mast cells was 28.0 ± 0.5, 35.3 ± 3.4, and 16.0 ± 0.8 in the SSO-feed, SSO-topical application, and SSO-feed/topical application groups, respectively (Fig. 4B and 4D). These results show that SSO significantly decreased epidermal thickening and mast cell infiltration in HDM-induced AD skins.

3.4. SSO decreases the expression of Th2 cytokines and inflammatory mediators in the AD skin

Th2 cytokines and inflammatory mediators are involved in AD. We measured the expression levels of IL-4, IL-5, IL-10, IL-13, IL-17A, and cyclooxygenase (COX)-2 in AD skin lesions using qRT-PCR (Fig. 5). The mRNA levels of IL-4, IL-5, IL-13, IL-17A, and COX-2 were significantly higher in skin of AD-positive group than in that of AD-negative group, and these levels were significantly decreased by treatment with SSO. Consistent with the AD symptom-scoring results of various SSO treatment regimens, the down-regulation of these cytokines was strongest in the SSO-feed/topical application group, while the SSO-feed or SSO-

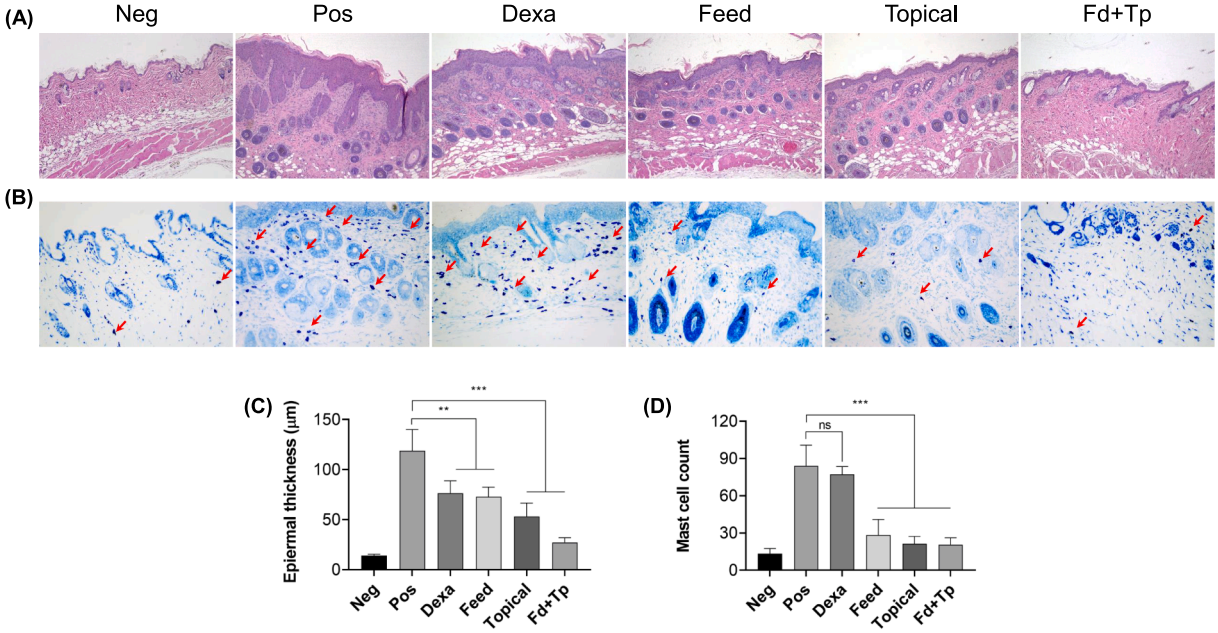


Fig. 4. Effects of SSO on the histology and infiltration of mast cells. (A) Representative histological images of the dorsal skin lesions. Hematoxylin and eosin staining was used to visualize epithelial hyperplasia (100X). Toluidine blue staining was used to visualize mast cell infiltration into the dermis (200X). (B) Mast cell infiltration is expressed as the average of total counts in three fields. The data are shown as the mean ± S.D. ns, not significant; *** $p < 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

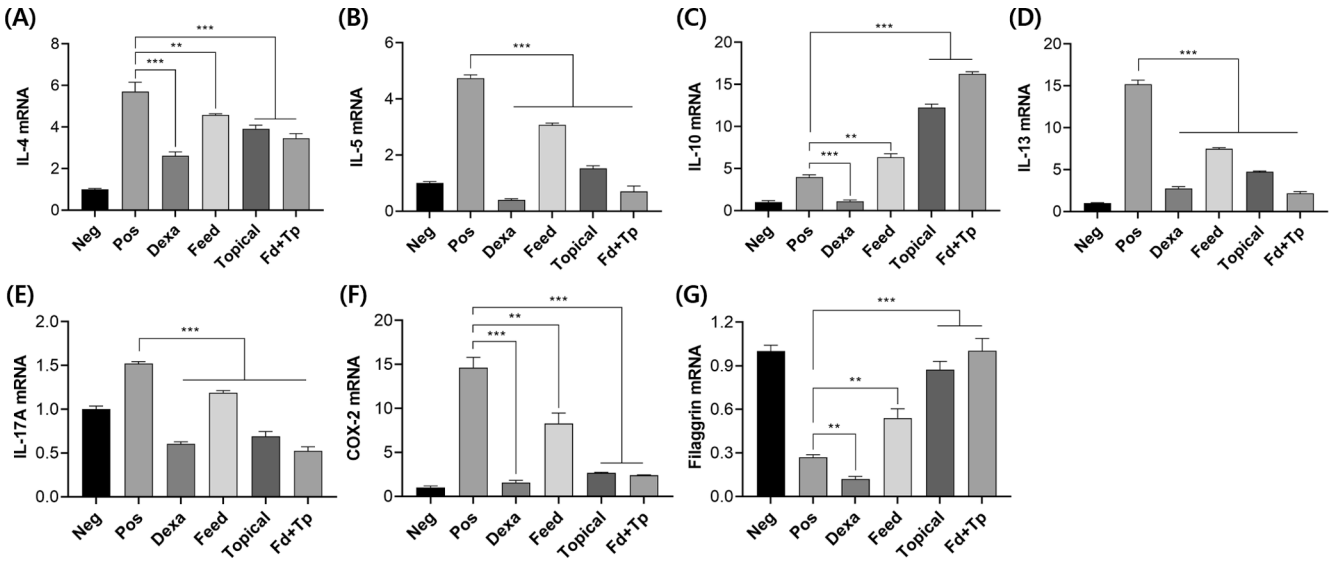


Fig. 5. Effects of SSO on AD-related cytokine and filaggrin gene expression in the dorsal skin. Total RNA was isolated from dorsal skin tissues, and the relative levels of (A) IL-4, (B) IL-5, (C) IL-10, (D) IL-13, (E) IL-17A, (F) COX-2, and (G) filaggrin were determined via qRT-PCR. All values were converted into fold changes compared with the expression level in the AD-negative group. The data are shown as the mean ± S.D. of three independent experiments. ** $p < 0.01$, *** $p < 0.001$. qRT-PCR, quantitative reverse transcription polymerase chain reaction.

topical application group exhibited moderate but significant down-regulating effects. However, SSO treatment increased the mRNA expression of IL-10, which plays a suppressive role in contact dermatitis and AD (Li & He, 2004; Boyman et al., 2012), and filaggrin, which is important for the alignment of keratin intermediate filaments, control of keratinocyte shape, and maintenance of epidermal texture (Moosbrugger-Martinz et al., 2022).

3.5. SSO increases the expression of tight junction-related proteins in the AD skin

We further performed Western blot analysis to investigate the effect of SSO on filaggrin expression at the protein level using total proteins prepared from AD skin tissues. Consistent with previous observations showing that SSO increased the expression of filaggrin mRNA in the AD skins, SSO also increased the amount of filaggrin protein in the AD skins (Fig. 6). The enhancing effect of SSO on filaggrin expression was strongest in the SSO-feed/topical application group, whereas the enhancing effect of SSO on filaggrin expression was moderate but significant in the SSO-feed or SSO-topical application group. To investigate the effect of SSO on the expression of other tight junction-related proteins, claudin-1, occludin, and ZO-1, additional Western blot analysis was performed using total proteins prepared from AD skin tissues. It was confirmed that SSO also increased the protein-level expression of claudin-1, occludin, and ZO-1 (Fig. 6). Consistent with other previous results, the enhancing effect of SSO on the expression of these proteins was found to be highest in the SSO-feed/topical application group.

3.6. SSO inhibits the production of IL-4, IL-5, and IL-13, while increases IL-10 production, from Th2 cells

We further investigated the effects of SSO on cytokine production by Th2 cells *in vitro*. The addition of SSO significantly inhibited the production of IL-4, IL-5, and IL-13 in a dose-dependent manner (Fig. 7A,B, D). In contrast, SSO significantly increased IL-10 in a dose-dependent manner (Fig. 7C). These results are in line with those obtained from qRT-PCR analysis of the dorsal skins.

4. Discussion

The pathogenesis of AD is multifactorial and involves the interplay between impaired skin barrier function, immune dysregulation, environmental triggers, and genetic defects (Li et al., 2021). Due to its complex pathogenic interplay, treatment of AD remains challenging (Bieber et al., 2010). Over the past decades, complementary and alternative medicines based on herbs or natural products have gained substantial attention for the management of AD (Vieira et al., 2016; Tumpang et al., 2018; Kwon et al., 2020; Hon et al., 2022, Mohd Kasim et al., 2022). Natural products may be low-cost, well-tolerated, and safe treatment options for AD. Here, we showed that SSO, an oil-in-water formula of edible sturgeon oil, is effective for the treatment of HDM-induced AD in NC/Nga mice. NC/Nga mice have a mutation on chromosome 9, which is related to increased IgE production and Th2-dominant inflammation, and many previous studies have used HDM extract-exposed NC/Nga mice as an AD animal model (Kohara et al., 2001; Yamamoto et al., 2007; Hwang et al., 2019; Kim et al., 2020; Choi et al., 2021; Umehara et al., 2023).

Our study demonstrates that SSO has potent therapeutic activity on HDM-induced AD symptoms in NC/Nga mice. Its therapeutic effects were confirmed by analyzing total IgE and HDM-specific IgE levels in the serum, mast cell infiltration, inhibition of Th2 cytokines, and increased filaggrin expression in the skin lesions, in addition to the visual observation of AD skin. Notably, the topical application of SSO to AD skin ameliorated AD lesions more effectively than dexamethasone ointment treatment. In addition, topical application of SSO did not cause weight loss, as was evident in the dexamethasone-treated group. The AD treatment effect of SSO was also observed in mice fed SSO, although it was weaker than that of the SSO-topical application regimen. The most potent therapeutic effect was observed in mice fed SSO along with topical SSO application.

Increased cutaneous production of Th2 cytokines, especially IL-4, IL-5 and IL-13, is universal in patients with AD (Ahn et al., 2020; Puar et al., 2021; Meng et al., 2021, Çetinarslan et al., 2023; Lee, 2023). Particularly, IL-4 and IL-13 are considered central to AD pathogenesis, because they play essential roles in Th2 cell differentiation and IgE production. IL-5 is a relatively eosinophil-specific cytokine that promotes eosinophil maturation and activation (Yamaguchi et al., 1988). We found that SSO inhibited IL-4, IL-5, and IL-13 production in the skin of AD-induced NC/

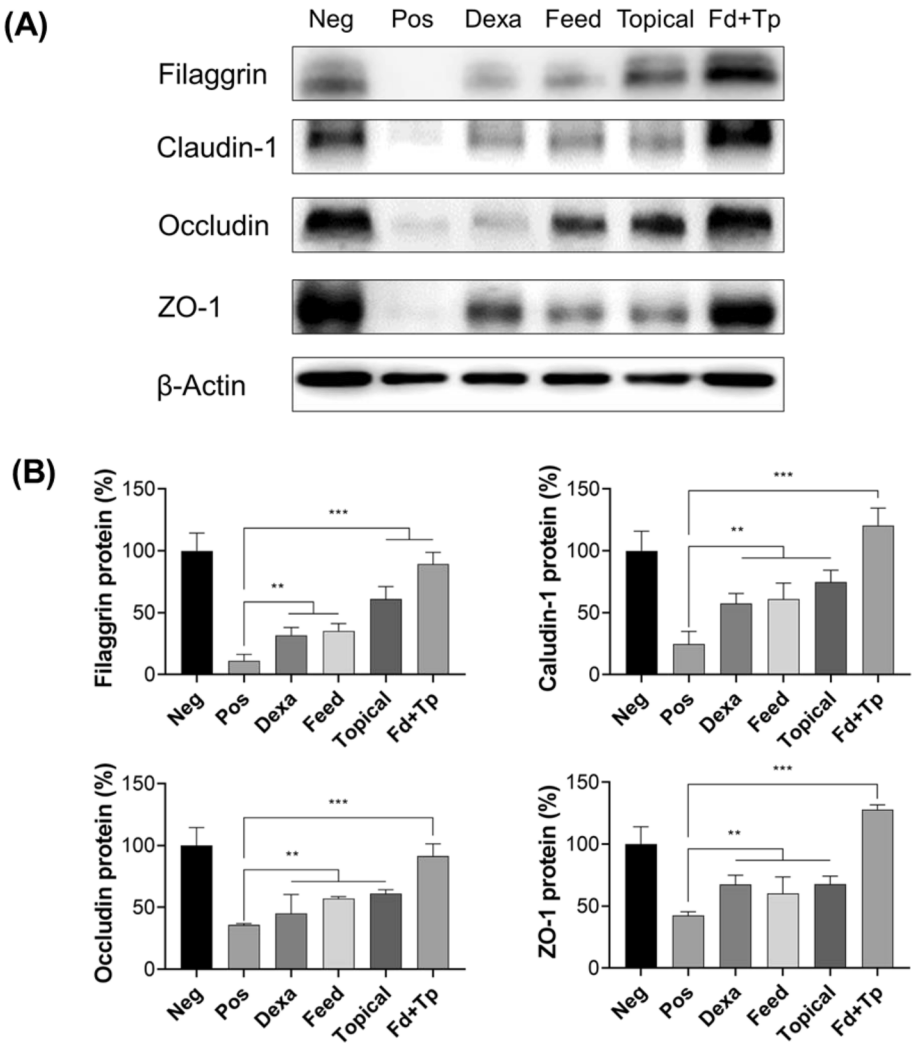


Fig. 6. Effect of SSO on the expression of filaggrin and other tight junction-related proteins in the AD skin. (A) Western blotting analysis of dorsal skin lysates showing expression levels of filaggrin, claudin-1, occludin, ZO-1, and β-Actin. (B) Bands were quantified and normalized using the EvolutionCapt edge software. The data are shown as the mean ± S.D. of three independent experiments. ** $p < 0.01$, *** $p < 0.001$.

Nga mice. In addition, SSO inhibited the production of the proinflammatory mediators IL-17A and COX-2 in AD skin, while increasing the production of IL-10, an immunosuppressive cytokine that alleviates inflammatory symptoms, including AD. To bolster the evidence that SSO inhibits Th2 cytokine production, we examined its effects using Th2 cells differentiated from naïve $CD4^+$ T cells *in vitro*. When added to Th2 cell cultures, SSO inhibited the production of IL-4, IL-5, and IL-13 in a dose dependent manner but increased that of IL-10. We speculate that SSO could be a promising material for AD therapy because it promotes the production of immunosuppressive IL-10 while inhibiting the production of AD-aggravating IL-4, IL-5, and IL-13.

SSO increased the production of filaggrin in the AD skin, which is an important structural protein responsible for the keratinization, moisturization, and antimicrobial peptide production of the skin (Hoyer et al., 2022; Moosbrugger-Martinz et al., 2022). Furthermore, SSO increased the production of tight junction-related proteins claudin-1, occludin, and ZO-1. Tight junctions play an important role in maintaining adhesion between cells and protecting them from the external environment (Katsarou et al., 2023). In AD skin, the skin barrier is weakened due to damage to tight junctions, and skin barrier dysfunction is a defining feature of AD skin (Beck et al., 2022). Since type 2 inflammation, characterized by overexpression of cytokines IL-4, IL-5, IL-13, and IL-31, is known to cause skin barrier dysfunction (Beck et al., 2022), the effect of SSO on restoring the levels of tight junction-related proteins may be mediated through the inhibition of type 2 inflammation by SSO. Additional studies are needed to clarify the exact mechanism of action of SSO in tight junctions.

Fish oil supplementation has been shown to have therapeutic efficacy on AD. It is an important source of PUFAs, which are indispensable components of the diet because they cannot be synthesized by the human body. PUFAs are composed of 18 or more carbon chains, with a double bond at six (ω-6 PUFAs) or three (ω-3 PUFAs) atoms away from the terminal methyl group. LA is the main ω-6 PUFA and can be elongated and desaturated to other bioactive ω-6 PUFAs, such as GLA and arachidonic acid. α-linolenic acid (ALA), which is an ω-3 PUFA, can be converted into eicosapentaenoic acid and subsequently into docosahexaenoic acid (DHA) (Kousparou et al., 2023). Beneficial effects of PUFAs in the management of AD have long been described. In clinical trials, LA-containing water-in-oil emulsions have been shown to significantly improve skin barrier dysfunction and the clinical severity of AD (Nasrollahi et al., 2018). Dietary supplementation with ALA and LA at a ratio of 4:1 or 1:1 has also been shown to be effective in treating IgE-mediated symptoms in 2,4-dinitrofluorobenzene-induced AD mice (Tang et al., 2020). In addition, GLA has been found to be effective without side effects in the treatment of patients with AD (Fiocchi et al., 1994; Andreassi et al., 1997; Simon et al., 2014). GLA is produced in the body from LA by the enzyme delta-6-desaturase, and the deficiency of this enzyme was observed in patients with AD, demonstrating the

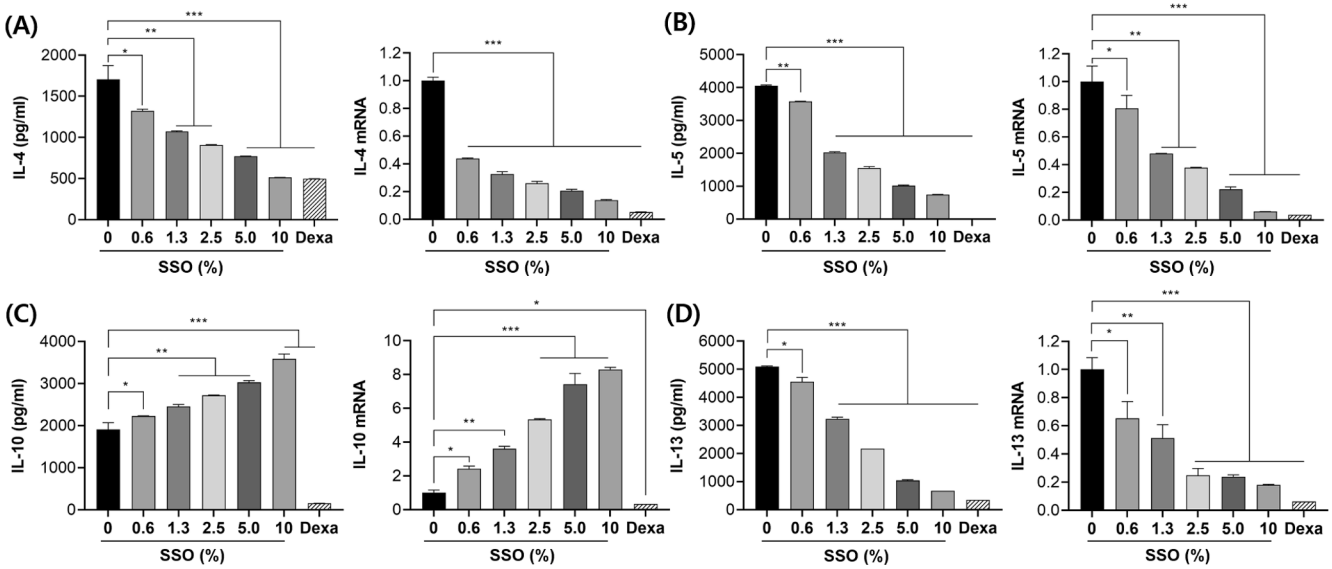


Fig. 7. Effect of SSO on Th2 cell differentiation and cytokine production *in vitro*. Naive $CD4^+$ T cells isolated from the spleens of C57BL/6 mice were induced to differentiate into Th2 cells by culturing in plates coated with anti-CD3 mAb and anti-CD28 mAb in the presence of IL-2, IL-4, and anti-IFN-γ mAb. Cells were harvested, washed, and stimulated by culturing in plates coated with anti-CD3 mAb and anti-CD28 mAb for 2 d in the presence of indicated concentrations of SSO. The culture supernatants were used for cytokine enzyme-linked immunosorbent assay, and the cell pellets were used for cytokine qRT-PCR. (A) IL-4, (B) IL-5, (C) IL-10, (D) IL-13. The data are shown as the mean ± S.D. of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

important roles of LA and GLA in AD management (Czumaj & Śledziński, 2020). Infants supplemented with DHA-rich formulas during the first year of life have a reduced incidence of AD during early childhood (Birch et al., 2010). Supplementation with ω-3 PUFAs during pregnancy was also shown to result in improvements in the clinical severity of allergic diseases in infants (Blümer & Renz, 2007; Furuhjelm et al., 2011). SSO contains various PUFAs including LA, ALA, GLA, and DHA. In addition, it contains palmitoleic acid, an ω-7 monounsaturated fatty acid, at the most abundant quantity. Palmitoleic acid has been shown to effectively improve the skin barrier function (Koh et al., 2023). Thus, it is reasonable to speculate that these fatty acids of SSO alone and in combination exert AD therapeutic activity, although identifying the exact entity responsible this activity remains a topic for further research. Notably, topical SSO application as well as oral SSO administration showed therapeutic effects on AD, and the most potent therapeutic efficacy was observed in mice treated with topical SSO application along with oral SSO administration. Why a combination of topical and oral administration can enhance the effectiveness of SSO also remains a topic of further research. However, we believe that topical application of SSO, an oil-in-water microemulsion that promotes skin penetration of PUFAs, will result in local concentration of PUFAs sufficient to exert AD therapeutic activity. This may be the reason for the improved efficacy seen in mice treated with topical SSO application in combination with oral SSO administration. To the best of our knowledge, this is the first study to show that topical application of fish oil exerts therapeutic effects on AD.

In summary, SSO, an oil-in-water formulation of sturgeon oil, may be a novel natural therapeutic product for treatment of AD. Topical application along with oral administration of SSO is effective in treating HDM-induced AD-like lesions in NC/Nga mice. SSO may exert its therapeutic effects on AD by suppressing the production of IgE, inflammatory mediators, and Th2 cytokines, while increasing the production of IL-10 and filaggrin.

CRediT authorship contribution statement

Hee-Sung Lee: Writing – original draft, Investigation. **Yong-Kwang Lee:** Resources. **Ji-Hyun Park:** Writing – original draft, Investigation. **Sang-Hyun Kim:** Investigation. **Chan-Su Park:** Writing – review & editing, Investigation. **Kyungjae Kim:** Writing – review & editing. **Chong-Kil Lee:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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