

Regulatory effects of chrysophanol, a bioactive compound of AST2017-01 in a mouse model of 2,4-dinitrofluorobenzene-induced atopic dermatitis

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ABSTRACT

The aim of this study is to determine whether AST2017-01 which consists of *Rumex crispus* and *Cordyceps militaris* would improve atopic dermatitis (AD). We analyzed anti-AD effects of AST2017-01 and chrysophanol, a bioactive compound of AST2017-01, using a 2,4-dinitrofluorobenzene-induced AD murine model. AST2017-01 and chrysophanol relieved clinical severity in AD-like skin lesions and significantly decreased scratching behavior. The thickness of epidermis and infiltration of inflammatory cells in AD-like skin lesions were reduced by AST2017-01 or chrysophanol. AST2017-01 and chrysophanol significantly suppressed the levels of histamine, immunoglobulin E, thymic stromal lymphopoietin (TSLP), interleukin (IL)-4, IL-6, and tumor necrosis factor- α in serum of AD mice. The protein levels of TSLP, intercellular adhesion molecule-1, and macrophage inflammatory protein 2 were significantly inhibited in the skin lesions. The mRNA expressions of TSLP, thymus and activation-regulated chemokine/CCL17, and C-C chemokine receptor 3 were inhibited in the skin lesions by AST2017-01 or chrysophanol. In addition, AST2017-01 and chrysophanol significantly suppressed the expressions and activities of caspase-1 in the skin lesions. Taken together, these results suggest that AST2017-01 has beneficial effects on AD and may be used as a health functional food in AD.

1. Introduction

Atopic dermatitis (AD) is the most common skin disease that affects millions of children and adults worldwide [1]. Inflammatory responses in AD are a consequence of a distinct microenvironment provided by various cytokines and chemokines [2]. Th1/Th2 cell dysregulation, immunoglobulin E (IgE) production, and mast cell hyperactivity play key roles in the evolution of the chronic, pruritic, and inflammatory dermatosis that characterize AD [3]. High interleukin (IL)-4 and IL-13 expressions which have been shown in serum of patients with AD are closely correlated with IgE levels [4,5]. IgE sensitizes local mast cells and causes histamine release from the mast cells and immediate allergic reactions in AD [6]. Thymic stromal lymphopoietin (TSLP) is involved in the initiation, development, and progression of AD both in mice and in humans [7]. The level of TSLP is regulated via caspase-1 pathway in mast cells [8]. The upregulation of caspase-1 is also related to the development of cutaneous inflammation [9].

Infiltrating of eosinophils and mast cells into atopic skin lesions

plays a critical role in the formation of atopic eczema [3,10]. Th2 cytokines, such as IL-4 and IL-13 which are major inducers of eotaxin-1 and proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α) contribute to infiltration of inflammatory cells in skin lesions of patients with AD [5,11]. Eotaxin-1 binds to C-C chemokine receptor 3 (CCR3) which is expressed on eosinophils, mast cells, and Th2 cells and induces chemotaxis and activation of mast cells and T cells in AD [12]. A Th2 chemokine, thymus and activation-regulated chemokine/CCL17 (TARC) actively participates in the pathogenesis of AD-like lesions [13]. Intercellular adhesion molecule-1 (ICAM-1) is crucial in initiation of inflammatory reaction in atopic skin and contributes to the development of Th2 cells [14]. Macrophage inflammatory protein 2 (MIP2) contributes to the leukocyte infiltration associated with AD [15].

AST2017-01 consists of Beopje processed-*Rumex crispus* and Beopje processed-*Cordyceps militaris* which have been commonly used as each functional food. *Rumex crispus* has been well known to have anti-microbial, anti-allergic, anti-oxidant properties [16,17]. *Cordyceps militaris* has been well known to alleviate inflammation, oxidative stress,

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and cancer [18,19]. Chrysophanol, a bioactive compound isolated from *Rumex crispus*, also has been known to have anti-inflammatory properties [20,21]. Jeong et al. [22] recently reported that AST2017-01 and chrysophanol have an anti-inflammatory effect on atopic dermatitis *in vitro* models. Furthermore, we investigated an anti-AD effect of AST2017-01 and chrysophanol using a 2,4-dinitrofluorobenzene-induced AD murine model in present study.

2. Materials and methods

2.1. Animals

We obtained female BALB/c mice from Dae-Han Experimental Animal Center (Eumsung, Republic of Korea). All procedures involving animals were approved by the animal care committee of Kyung Hee University [KHUASP(SE)-18-022]. All mice were acclimatized to standard laboratory conditions for a week prior to AD experiments.

2.2. Sensitization with DNFB

Acetone (100 μ l) was topically applied to shaved abdominal skins of mice. A week later, acetone (50 μ l) was applied to shaved dorsal skin twice a week for 3 weeks as a vehicle group. For DNFB (Sigma Chemical Co., St. Louis, MO, USA) sensitization, 0.15% DNFB in acetone (100 μ l) was topically applied to shaved abdominal skins of mice. A week later, 0.15% DNFB (50 μ l) was topically applied to shaved dorsal skins twice a week for 3 weeks as a DNFB control group. AST2017-01 (Gahwa Well Food Co., Chungbuk, Republic of Korea, 0.5, 5, and 50 mg/kg), chrysophanol (Sigma Chemical Co., 0.06 mg/kg), or dexamethasone (Sigma Chemical Co., DEX, 39 μ g/kg) was orally administered to DNFB-applied mice in a volume of 0.1 ml/10 g by a sonde 3 times a week for 3 weeks ($n = 5$ /group). AST2017-01, chrysophanol, and DEX were prepared according to our previous report [22]. Clinical features were observed after the last DNFB application. The duration of the scratching behavior was measured for 10 min after the last DNFB application with reference to previous reports [23,24]. After the scratching test, serum and dorsal skin samples were collected 4 h after the last DNFB application.

2.3. Histological analysis of AD

For histological analysis, biopsies of skin lesions were fixed with formaldehyde and embedded in paraffin. The sections were cut at a thickness of 4 μ m, dewaxed, cleared in xylene, and hydrated. Each section was stained with hematoxylin and eosin (H&E) to visualize epidermal thickness and eosinophils or with toluidine blue to visualize mast cells. We counted the numbers of eosinophils and mast cells in five randomly visualized images.

2.4. Histamine assay

The histamine levels in serum of AD mice were measured with *o*-phthaldialdehyde (OPA) spectrofluorometric procedure. The fluorescent intensity was evaluated at 440 nm (excitation at 360 nm) in spectrofluorometer.

2.5. Cytokines assay

The levels of IgE (BD Pharmingen, San Diego, CA, USA), TSLP (R&D Systems, Minneapolis, MN, USA), IL-4 (BD Pharmingen), IL-6 (BD Pharmingen), IL-13 (BD Pharmingen), TNF- α (BD Pharmingen), and IFN- γ (BD Pharmingen) in serum and levels of TSLP, ICAM-1 (R&D Systems), and MIP2 (R&D Systems) in DNFB-induced skin lesions were analyzed using a sandwich ELISA method according to manufacturer's protocols, as previously described [25].

2.6. PCR analysis

We isolated total RNA from DNFB-induced skin lesions with an easy-BLUE™ RNA extraction kit (iNtRON Biotech, Republic of Korea). We performed reverse transcription PCR with the following primers: TSLP (5' TGC AAG TAC TAG TAC GGA TGG GG C 3'; 5' GGA CTT CTT GTG CCA TTT CCT GAG 3'); TARC (5' CAG GAA GTT GGT GAG CTG GTA TA 3'; 5' TTG TGT TCG CCT GTA GTG CAT A 3'); CCR3 (5' TGC TAC TAT CAC CAG TAT CAT TAC C 3'; 5' GCT TGT TCT TTC CAT TTT CTC ACC 3'); GAPDH (5' GGC ATG GAC TGT GGT CAT GA 3'; 5' TTC ACC ACC ATG GAG AAG GC 3'). The annealing temperatures were 62 °C for TSLP; 55 °C for TARC and CCR3; 60 °C for GAPDH. PCR products were separated on a 1.5% agarose gel and visualized by ethidium bromide. Quantitative real-time PCR was performed using a SYBR Green Master Mix and the detection of mRNA was analyzed using an ABI StepOne real time PCR System (Applied Biosystems, Foster City, CA, USA). We used the following primers for real-time PCR; TSLP (5' AGG CTA CCC TGA AAC TGA GAG AAA 3'; 5' GCG ACA GCA TGG TTC TTC TCA 3'); TARC (5' TTG TGT TCG CCT GTA GTG CAT A 3'; 5' CAG GAA GTT GGT GAG CTG GTA TA 3'); CCR3 (5' AAA GCT GAT ACC AGA GCA CTG 3'; 5' CCA AGA GGC CCA CAG TGA AC 3'); GAPDH (5' GGC AAA TTC AAC GGC ACA 3'; 5' GTT AGT GGG GTC TCG CTC CTG 3'). Each level of target mRNA was normalized to the level of GAPDH. Results were analyzed using the $\Delta\Delta$ CT method.

2.7. Western blotting analysis

Tissues obtained from skin lesions of AD mice were homogenized with homogenization buffer in a motor-driven homogenizer. And then protein aliquots were resolved through 12% SDS-PAGE. After electrophoresis, the proteins were transferred to nitrocellulose membranes. The membranes were blocked with phosphate buffered saline with Tween 20 containing 5% nonfat dry milk and incubated with anti-caspase-1 and GAPDH antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and secondary antibodies (Santa Cruz Biotechnology). The specific bands of target proteins were visualized by an enhanced chemiluminescence assay according to manufacturer's protocols (Amersham Co. Newark, NJ, USA).

2.8. Caspase-1 assay

Caspase-1 activity in DNFB-induced skin lesions was measured using a caspase-1 assay kit according to manufacturer's protocols (R&D System).

2.9. Statistical analysis

All results are shown as mean $\pm$ S.E.M. Statistical analysis was performed by an independent *t*-test and ANOVA with Tukey *post hoc* test using IBM SPSS v23 statistics software (IBM, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Regulatory effects of AST2017-01 and chrysophanol on clinical symptoms of AD-like skin lesions

First, we examined regulatory effects of AST2017-01 and chrysophanol on clinical symptoms of AD-like lesions. DNFB application induced striking erythema, hemorrhage, and erosion in AD-like skin lesions, whereas AST2017-01 and chrysophanol relieved these symptoms (Fig. 1A). AST2017-01 (50 mg/kg) and chrysophanol significantly decreased the duration of scratching behavior (Fig. 1B, $P < 0.05$). DEX

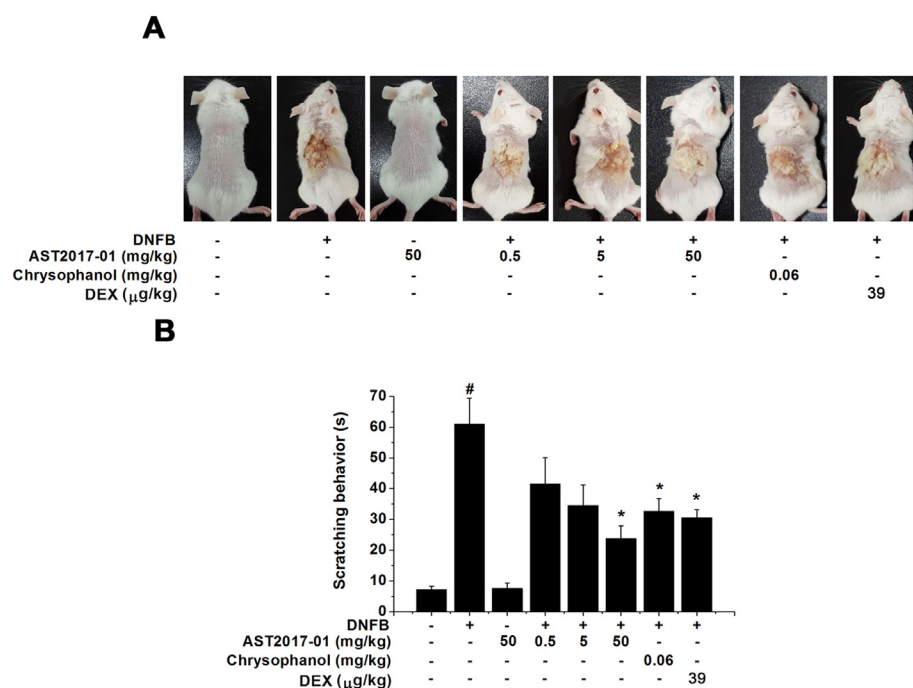


Fig. 1. Regulatory effects of AST2017-01 and chrysophanol on clinical symptoms of AD-like skin lesions. (A) Each clinical feature was observed after the last DNFB application. (B) The duration of scratching behavior was assessed for 10 min. Each datum represents the mean $\pm$ S.E.M. [#] $P < 0.05$ vs a vehicle group. ^{*} $P < 0.05$ vs a DNFB control group. $n = 5$ /group. DEX, dexamethasone.

also relieved the clinical symptoms (Fig. 1A) and scratching behavior (Fig. 1B, $P < 0.05$).

3.2. Regulatory effects of AST2017-01 and chrysophanol on epidermis thickness and infiltration of inflammatory cells of AD-like skin lesions

Next, we examined whether AST2017-01 and chrysophanol could attenuate epidermis thickness of AD-like skin lesions. As shown in Fig. 2(A, upper panel), DNFB application increased epidermis thickness of the skin lesions, whereas AST2017-01 and chrysophanol decreased the epidermis thickness. And AST2017-01 and chrysophanol significantly decreased the infiltrations of eosinophils (A, middle panel) and mast cells (A, lower panel) in AD-like lesions (Fig. 2A and B, $P < 0.05$). DEX also decreased the epidermis thickness (Fig. 2A) and the infiltrations of eosinophils and mast cells (Fig. 2A and B, $P < 0.05$).

3.3. Regulatory effects of AST2017-01 and chrysophanol on mediators of atopic inflammation in serum of DNFB-induced AD mice

We examined whether the levels of histamine and IgE in serum of DNFB-induced AD mice could be regulated by AST2017-01 and chrysophanol. The levels of histamine and IgE increased by DNFB application were significantly decreased by AST2017-01 (50 mg/kg) or chrysophanol ($P < 0.05$, Table 1). Further, we tested regulatory effects of AST2017-01 and chrysophanol on inflammatory cytokines in the serum of the DNFB-applied mice. AST2017-01 significantly decreased the levels of TSLP, IL-4, IL-6, IL-13, and TNF- α in the serum ($P < 0.05$, Table 1). Chrysophanol significantly decreased the levels of TSLP, IL-4, IL-6, and TNF- α in the serum of the DNFB-applied mice ($P < 0.05$, Table 1). DEX also significantly decreased the levels of histamine, IgE, TSLP, IL-4, IL-6, IL-13, and TNF- α in the serum of the DNFB-applied mice ($P < 0.05$, Table 1). However, IFN- γ levels were not significantly

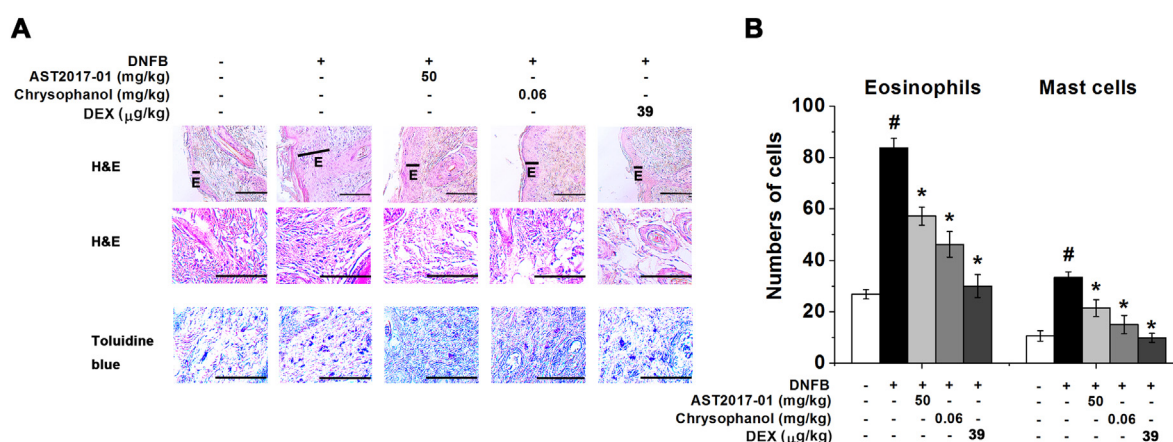


Fig. 2. Regulatory effects of AST2017-01 and chrysophanol on epidermis thickness and infiltration of inflammatory cells of AD-like skin lesions. (A, upper) Epidermal thickness was analyzed in five randomly visualized images by H&E staining at 200 $\times$ magnification. (A, middle) Eosinophils and (A, lower) mast cells were analyzed in five randomly visualized images by H&E staining and toluidine blue staining respectively. (Scale bar = 150 μ m). These images are representative. (B) The numbers of eosinophils and mast cells were counted in five randomly visualized images. Each datum represents the mean $\pm$ S.E.M. [#] $P < 0.05$ vs a vehicle group. ^{*} $P < 0.05$ vs a DNFB control group. $n = 5$ /group. E, epidermis; DEX, dexamethasone. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Regulatory effects of AST2017-01 and chrysophanol on mediators of atopic inflammation in serum of DNFB-induced AD mice.

		Vehicle	DNFB	DNFB				Chrysophanol (mg/kg)	DEX (μg/kg)
				AST2017-01 (mg/kg)					
				50	0.5	5	50	0.06	39
Serum	Histamine	31.60 ± 2.46	53.95 ± 2.92 [#]	28.98 ± 3.20	44.98 ± 2.74	41.46 ± 1.04	34.97 ± 1.07 [*]	34.87 ± 1.02 [*]	34.85 ± 1.83 [*]
	IgE	8.84 ± 1.00	16.55 ± 0.70 [#]	6.17 ± 0.58	17.78 ± 2.67	11.28 ± 1.48	9.62 ± 0.44 [*]	9.40 ± 0.65 [*]	9.24 ± 0.64 [*]
	TSLP	1.07 ± 0.07	2.99 ± 0.29 [#]	0.86 ± 0.08	2.29 ± 0.19	1.47 ± 0.05 [*]	1.27 ± 0.10 [*]	2.05 ± 0.13 [*]	1.35 ± 0.13 [*]
	IL-4	11.17 ± 0.30	17.01 ± 1.44 [#]	13.32 ± 1.21	14.06 ± 0.65	12.20 ± 0.65	11.77 ± 0.25 [*]	9.94 ± 0.72 [*]	11.18 ± 0.52 [*]
	IL-6	0.68 ± 0.30	3.64 ± 1.02 [#]	0.21 ± 0.06	1.36 ± 0.74 [*]	0.50 ± 0.14 [*]	0.42 ± 0.09 [*]	0.41 ± 0.08 [*]	0.85 ± 0.24 [*]
	IL-13	1.80 ± 0.10	2.68 ± 0.17 [#]	1.71 ± 0.08	2.39 ± 0.11	1.96 ± 0.11	1.57 ± 0.16 [*]	1.99 ± 0.13	1.60 ± 0.18 [*]
	TNF-α	51.13 ± 3.75	79.32 ± 13.27 [#]	54.08 ± 2.39	49.95 ± 4.04 [*]	47.60 ± 6.16 [*]	42.73 ± 2.89 [*]	36.19 ± 4.65 [*]	28.40 ± 3.55 [*]
	IFN-γ	1.10 ± 0.03	0.94 ± 0.04	1.16 ± 0.05	1.08 ± 0.04	1.13 ± 0.05	1.16 ± 0.10	1.25 ± 0.13	0.99 ± 0.04

Histamine levels (ng/ml) in serum were measured with OPA spectrofluorometric procedure as described in the [Materials and methods](#) section. The levels of IgE (ng/ml), TSLP (ng/ml), IL-4 (ng/ml), IL-6 (ng/ml), IL-13 (ng/ml), TNF-α (ng/ml), and IFN-γ (ng/ml) in serum were determined using ELISA method. Numerical results are reported as mean ± SEM. n = 5.

[#] *P* < 0.05 vs a vehicle group. DEX, dexamethasone.

^{*} *P* < 0.05 vs a DNFB control group.

regulated by AST2017-01, chrysophanol, or DEX ([Table 1](#)).

3.4. Regulatory effects of AST2017-01 and chrysophanol on proteins levels of mediators of atopic inflammation in AD-like skin lesions

Next, we determined the levels of TSLP, ICAM-1, and MIP2 in AD-like skin lesions. As shown in [Table 2](#), DNFB significantly increased the protein expressions of TSLP, ICAM-1, and MIP2, whereas AST2017-01 and chrysophanol significantly inhibited the expressions of TSLP, ICAM-1, and MIP2 in the skin lesions (*P* < 0.05). DEX also significantly inhibited the expressions of TSLP, ICAM-1, and MIP2 in the skin lesions (*P* < 0.05).

3.5. Regulatory effects of AST2017-01 and chrysophanol on mRNA levels of mediators of atopic inflammation in AD-like skin lesions

Next, we examined whether AST2017-01 and chrysophanol could regulate mRNA expressions of mediators of atopic inflammation in AD-like skin lesions using reverse transcription PCR and real-time PCR analyses. First, the reverse transcription PCR analysis showed that the mRNA expressions of TSLP, TARC, and CCR3 were up-regulated by DNFB application, whereas AST2017-01 and chrysophanol significantly decreased the mRNA expressions of TSLP, TARC, and CCR3 up-regulated by DNFB application in the skin lesions (*P* < 0.05, [Fig. 3A](#) and [B](#)). Real-time PCR analysis also showed that AST2017-01 and chrysophanol significantly decreased the mRNA expressions of TSLP, TARC, and CCR3 in the skin lesions (*P* < 0.05, [Fig. 3C](#)). DEX also significantly decreased the mRNA expressions of TSLP, TARC, and CCR3 (*P* < 0.05, [Fig. 3](#)).

Table 2

Regulatory effects of AST2017-01 and chrysophanol on proteins levels of mediators of atopic inflammation in AD-like skin lesions.

		Vehicle	DNFB	DNFB				Chrysophanol (mg/kg)	DEX (μg/kg)
				AST2017-01 (mg/kg)					
				50	0.5	5	50	0.06	39
Lesional tissue	TSLP	0.14 ± 0.03	0.68 ± 0.08 [#]	0.16 ± 0.03	1.06 ± 0.11	0.65 ± 0.04	0.29 ± 0.03 [*]	0.32 ± 0.05 [*]	0.33 ± 0.06 [*]
	ICAM-1	6.82 ± 0.47	12.38 ± 0.56 [#]	7.02 ± 0.37	10.10 ± 0.57	6.80 ± 0.46 [*]	5.06 ± 0.73 [*]	4.00 ± 0.48 [*]	5.11 ± 0.47 [*]
	MIP2	1.16 ± 0.21	4.23 ± 0.58 [#]	1.37 ± 0.21	2.03 ± 0.14 [*]	1.17 ± 0.05 [*]	0.70 ± 0.11 [*]	0.80 ± 0.04 [*]	1.53 ± 0.17 [*]

The levels of TSLP, ICAM-1, and MIP2 in skin lesions were determined using ELISA method. All parameters measured in skin lesions homogenate were presented as a ratio to the total protein level. Numerical results are reported as mean ± SEM. n = 5. DEX, dexamethasone.

[#] *P* < 0.05 vs a vehicle group.

^{*} *P* < 0.05 vs a DNFB control group.

3.6. Regulatory effects of AST2017-01 and chrysophanol on caspase-1 activity in AD-like skin lesions

Caspase-1 mainly affects the secretion of proinflammatory cytokines [26]. In addition, TSLP level was increased through caspase-1 activation in an allergic murine model [27]. Finally, we investigated whether AST2017-01 and chrysophanol would inhibit atopic inflammation by regulating caspase-1 signaling in AD-like skin lesions. As shown in [Fig. 4A](#) and [B](#), AST2017-01 and chrysophanol significantly suppressed the protein levels of caspase-1 in the skin lesions (*P* < 0.05). In addition, AST2017-01 and chrysophanol significantly reduced the caspase-1 activities in the skin lesions ([Fig. 4C](#), *P* < 0.05). DEX also significantly decreased the protein levels and activities of caspase-1 ([Fig. 4](#), *P* < 0.05).

4. Discussion

The present study shows that AST2017-01 and chrysophanol relieved clinical severity in AD-like skin lesions and scratching behavior of DNFB-induced AD mice. AST2017-01 and chrysophanol decreased the thickness of epidermis and infiltration of inflammatory cells in the skin lesions. AST2017-01 and chrysophanol suppressed the levels of histamine, IgE, TSLP, IL-4, IL-6, and TNF-α in serum and protein levels of TSLP, ICAM-1, and MIP2 in the skin lesions. The mRNA expressions of TSLP, TARC, and CCR3 in the skin lesions were inhibited by AST2017-01 or chrysophanol. In addition, AST2017-01 and chrysophanol suppressed the activities of caspase-1 in the skin lesions.

Intense itching, erythema, and dryness as skin symptoms were observed in skin lesions of AD [28]. The high histamine and IgE levels cause skin inflammation and itching [29]. Also, histamine, IgE, IL-4, IL-

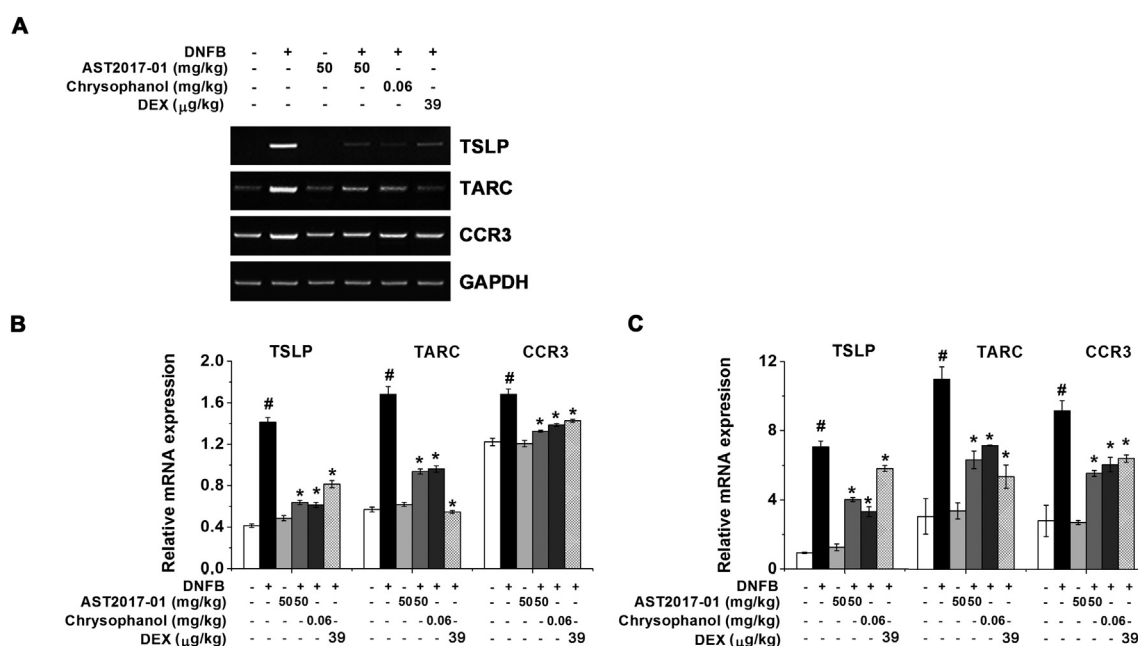


Fig. 3. Regulatory effects of AST2017-01 and chrysophanol on mRNA levels of mediators of atopic inflammation in AD-like skin lesions. (A) The mRNA expressions of TSLP, TARC, and CCR3 were analyzed with reverse transcription PCR method. (B) Each mRNA expression was quantified by densitometry. (C) The mRNA expressions of TSLP, TARC, and CCR3 were analyzed with real-time PCR method. Each datum represents the mean $\pm$ S.E.M. # $P < 0.05$ vs a vehicle group. * $P < 0.05$ vs a DNFB control group. $n = 5$ /group. TARC, thymus and activation-regulated chemokine/CCL17; CCR3, C-C chemokine receptor 3; DEX, dexamethasone.

6, and TNF- α levels significantly increased in serum of AD patients than healthy controls [30,31]. TSLP was highly expressed in serum and biopsies of skin lesions from patients with AD [32,33]. Albrecht and Dittrich [34] reported that an anti-histamine therapy reduced itch severity and duration in patients with AD. Also, Guttman-Yassky et al. [35] reported that inhibitors of IgE, Th2 cytokines, and pro-inflammatory cytokines can be used to treat AD. Our previous study reported that the inflammatory responses and severity of AD were reduced in TSLP-deficient mice [36]. In the present study, we showed that AST2017-01 and chrysophanol suppressed the clinical severity in AD-like skin lesions and scratching behavior of AD mice. Also, AST2017-01 and chrysophanol reduced the levels of histamine, IgE, TSLP, IL-4, IL-6, and TNF- α in serum of AD mice. Thus, we can infer that AST2017-01 and chrysophanol have an anti-AD effect by inhibiting AD-related biomarkers.

Chemokines and their receptors play a critical role in AD orchestrating the initiation and exacerbation of inflammatory responses [37]. Chemokines induce infiltrations of eosinophils and mast cells in skin with AD [38,39]. The levels of CCR3, ICAM-1, and MIP2 were elevated in skin of patients with AD or AD-like skin lesions of DNFB-applied mice [14,15,40]. TARC increased highly in skin lesions of DNFB-applied mice is involved in the infiltration of Th2 cells into skin [41]. Topical tacrolimus treatment has been effective in the treatment of AD by inhibiting infiltration of cytokines-expressing inflammatory cells [42]. Lim et al. [43] reported that an herbal medicine (*Alpinia katsumadai* Hayata) has anti-AD effects, by reducing erosion, hyperplasia of epidermis, and inflammatory cells infiltration in skin lesions of a murine model of AD. In the present study, we showed that AST2017-01 and chrysophanol decreased the infiltrations of eosinophils and mast cells in the skin lesions, suppressing the levels of ICAM-1, MIP2, TARC, and CCR3. Thus, we suggest that AST2017-01 and chrysophanol have an anti-AD effect by inhibiting infiltration of inflammatory cells through regulating chemokines.

The increase in caspase-1 level induces inflammation [9]. Mice overexpressing caspase-1 showed spontaneous development of AD-like inflammatory skin lesions with high levels of histamine and IgE in

serum [44]. DNFB-induced murine contact hypersensitivity was suppressed in caspase-1-deficient mice [45]. Lack of caspase-1 reduced the number of eosinophils and expression of Th2 cytokines at allergic reaction exacerbation [27]. Joosten et al. [46] reported that inhibition of caspase-1 might be successful in treatment of inflammatory diseases of humans. In our previous report, AST2017-01 and chrysophanol suppressed the TSLP levels through inhibition of caspase-1 activation *in vitro* models, suggesting that AST2017-01 acts as an anti-inflammatory herbal medicine. Furthermore, in the present study, we showed that AST2017-01 and chrysophanol suppressed the protein levels and activities of caspase-1 in the AD-like skin lesions. Thus, we suggest that AST2017-01 and chrysophanol have an anti-AD effect *via* regulating caspase-1 pathways.

In conclusion, it is noteworthy that AST2017-01 and chrysophanol suppressed the clinical symptoms of a DNFB-induced AD murine model. AST2017-01 and chrysophanol reduced the levels of histamine, IgE, inflammatory cytokines, and chemokines with decreases in infiltration of inflammatory cells in AD mice. In addition, AST2017-01 and chrysophanol reduced the caspase-1 levels in the skin lesions. Therefore, AST2017-01 may be useful in the treatment of AD. However, we suggest that further studies are needed to determine whether AST2017-01 and chrysophanol have regulatory effects on AD human models.

Conflict of interest disclosure

None declared.

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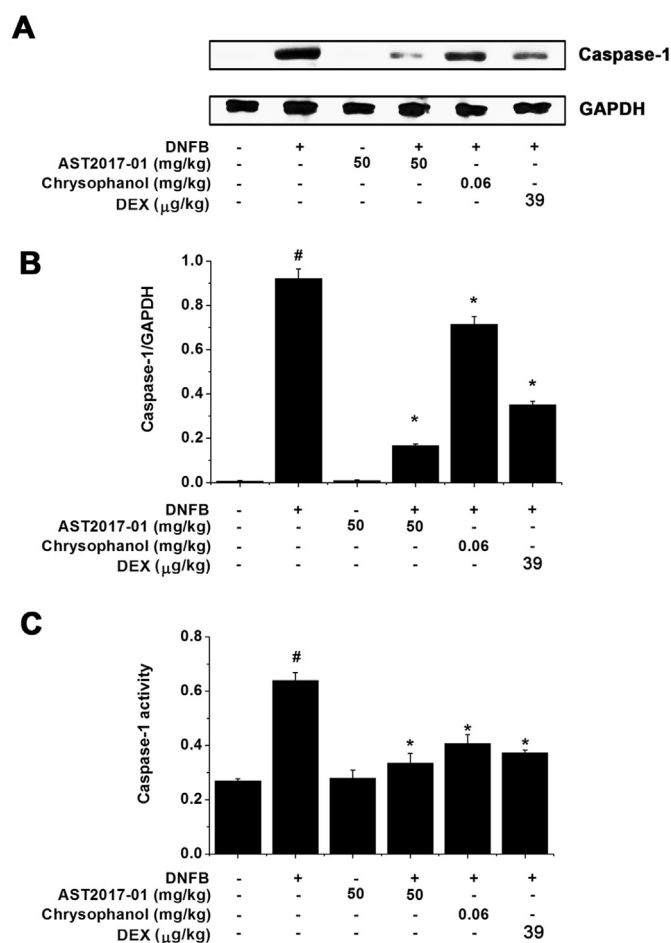


Fig. 4. Regulatory effects of AST2017-01 and chrysophanol on caspase-1 activity in AD-like skin lesions. (A) Caspase-1 protein levels were evaluated with Western blotting. (B) The expressions were quantified by densitometry. (C) Caspase-1 activities in skin lesions homogenate were evaluated with a caspase-1 assay kit. Each datum represents the mean $\pm$ S.E.M. [#] $P < 0.05$ vs a vehicle group. ^{*} $P < 0.05$ vs a DNFB control group. $n = 5$ /group. DEX, dexamethasone.

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None.

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