

Xanthone suppresses allergic contact dermatitis *in vitro* and *in vivo*

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ABSTRACT

Xanthone is a phenolic compound found in a few higher plant families; it has a variety of biological activities, including antioxidant, anti-inflammatory, and anticancer properties. However, the molecular and cellular mechanisms underlying the activity of xanthone in allergic contact dermatitis (ACD) remain to be explored. Therefore, this study aimed to investigate the regulatory effects of xanthone in ACD in human keratinocytes (HaCaT cell), and human mast cell line (HMC-1 cell) *in vitro* and in an experimental murine model. The results demonstrated that treatment with xanthone reduced the production of pro-inflammatory cytokines and chemokines including interleukin (IL)-1 β , IL-6, IL-8, and expression of chemokines thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) in tumor necrosis factor (TNF)- α and interferon (IFN)- γ -stimulated HaCaT cells. Xanthone also suppressed the production of pro-inflammatory cytokines, chemokines, and allergic mediators in phorbol myristate acetate/A23187 calcium ionophore (PMACI)-stimulated HMC-1 cells. Xanthone significantly suppressed the phosphorylation of mitogen-activated protein kinases (MAPKs) and nuclear factor-kappa B (NF- κ B) and activation of caspase-1 signaling pathway *in vitro* model. Additionally, xanthone administration alleviated 2,4-dinitrofluorobenzene (DNFB)-induced atopic dermatitis like-skin lesion by reducing the serum levels of immunoglobulin E (IgE), histamine, and pro-inflammatory cytokines and suppressing MAPKs phosphorylation. Xanthone administration also inhibited mortality due to compound 48/80-induced anaphylactic shock and suppressed the passive cutaneous anaphylaxis (PCA) reaction mediated by IgE. Collectively, these results suggest that xanthone has a potential for use in the treatment of allergic inflammatory diseases.

1. Introduction

Allergic contact dermatitis (ACD) is a highly prevalent inflammatory disease of the skin that is characterized by skin allergic signs and symptoms including redness, edema, and pruritus [1]. Allergic disorders, such as ACD, anaphylaxis, and urticaria, affect approximately 25% of the population of the developed world [2]. The occurrence of ACD correlates with the expression and release of pro-inflammatory mediators from keratinocytes, mast cells, and other immune cells [3]. The pathophysiology of ACD is primarily studied using animal models in which skin inflammation is induced by the repeated topical application of haptens, such as 2,4-dinitrofluorobenzene (DNFB) and oxazolone (Ox) [4,5].

Keratinocyte releases immunomodulatory mediators allow immune cells to enter the site of inflammation in the skin [6]. ACD is commonly characterized by the infiltration of Th2-type lymphocytes through the

skin lesions and is known to be associated with high levels of chemokines [7,8]. The mast cells play crucial roles in allergic inflammation, as they constitute the effector cells of the immediate immunoglobulin E (IgE)-driven hypersensitivity reaction and the resulting in the release of histamine and other inflammatory mediators [9–11]. Thymic stromal lymphopoietin (TSLP) is mainly produced by mast cells and plays a critical role in the development of allergic diseases [12]. Numerous studies have demonstrated that pro-inflammatory cytokines and chemokines promote inflammation, leukocyte infiltration, and granuloma formation [13–17]. The inhibition of pro-inflammatory mediators could effective therapeutic strategies for ACD.

Mitogen-activated protein kinases (MAPKs), such as the p42/p44 extracellular regulated kinases (ERKs), the c-Jun N terminal kinases (JNKs) and the p38 mitogen-activated protein kinase (p38 MAPK), during the immune response in ACD is associated with the activation of nuclear factor kappa B (NF- κ B). NF- κ B plays a crucial role in the

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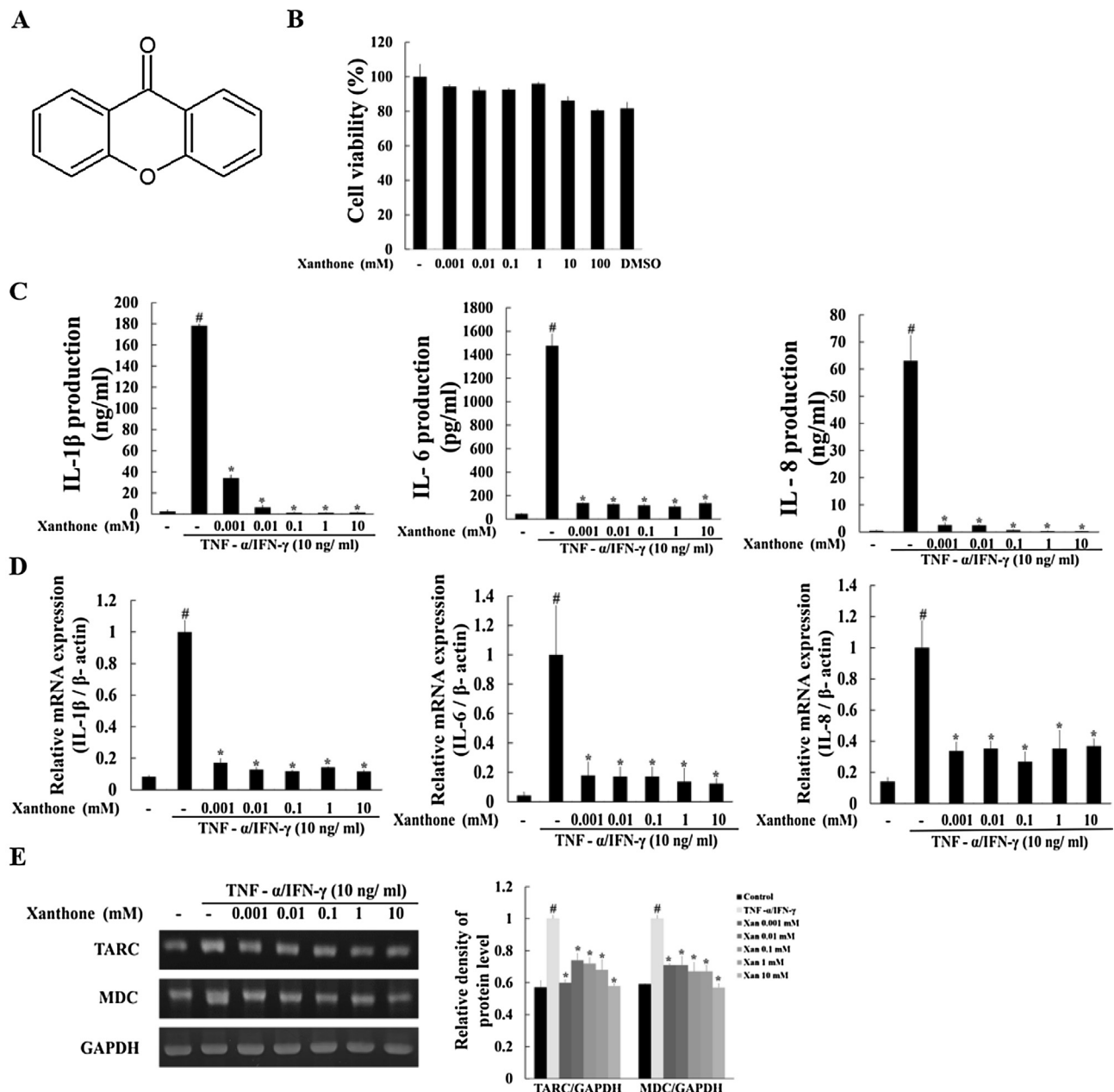


Fig. 1. Effects of xanthone on the production of pro-inflammatory mediators in TNF- α /IFN- γ -stimulated HaCaT cells. (A) Structure of xanthone. (B) Cell viability. (C) The production levels of IL-1 β , IL-6, and IL-8. (D) The mRNA expression of IL-1 β , IL-6, and IL-8. (E) The mRNA expression of TARC and MDC. The data represent the mean $\pm$ S.E.M. (# p < 0.05 vs. control group, * p < 0.05 vs. TNF- α /IFN- γ -stimulated group).

Table 1
Sequences of the primers used in RT-PCR.

Genes	Primers	Sequence (5'-3')
TARC (h)	Forward	ATGGCCCCACTGAAGATGCT
	Reverse	TGAACACCAACGGTGGAGGT
MDC (h)	Forward	GATGGCTCGCCTACAGACT
	Reverse	GCAGGGAGGGAGGCAGAGGA
GAPDH (h)	Forward	ATTGTTGCCATCAATGACCC
	Reverse	AGTAGAGGCAGGGATGATGT

expression of several genes involved in immune and inflammatory responses, including allergies [18–20]. The activation of caspase-1 induces the production of pro-inflammatory cytokines via the nucleus translocation of NF- κ B [20]. Therefore, the inhibitions of MAPKs, NF- κ B, and caspase-1 are the strategy to reduce the severity of

Table 2
Sequences of the primers used in real-time RT-PCR.

Genes	Primers	Sequence (5'-3')
L-1 β (h)	Forward	AAACAGATGAAGTGCTCTT
	Reverse	TGGAGAACACCACTTGTTC
IL-6 (h)	Forward	TGTCCTGCAGCCACTGGTTC
	Reverse	AAGCCAGAGCTGTGCAGATGAGTA
IL-8 (h)	Forward	TTTGCTTGAAGTTTCACTGGCATC
	Reverse	ACACTGGGCCAACACAGAAATTA
TNF- α (h)	Forward	CAGCCTTGGCCCTTGAAGA
	Reverse	GACAAGCCTGTAGCCCATGTTGTA
TSLP (h)	Forward	TATGAGTGGGACCAAAAGTACCG
	Reverse	GGGATTGAAGTTTAGGCTCTGG
β -actin (h)	Forward	ATGGAGCCACCGATCCACA
	Reverse	ATTGCCGACAGGATGCAAGAAG

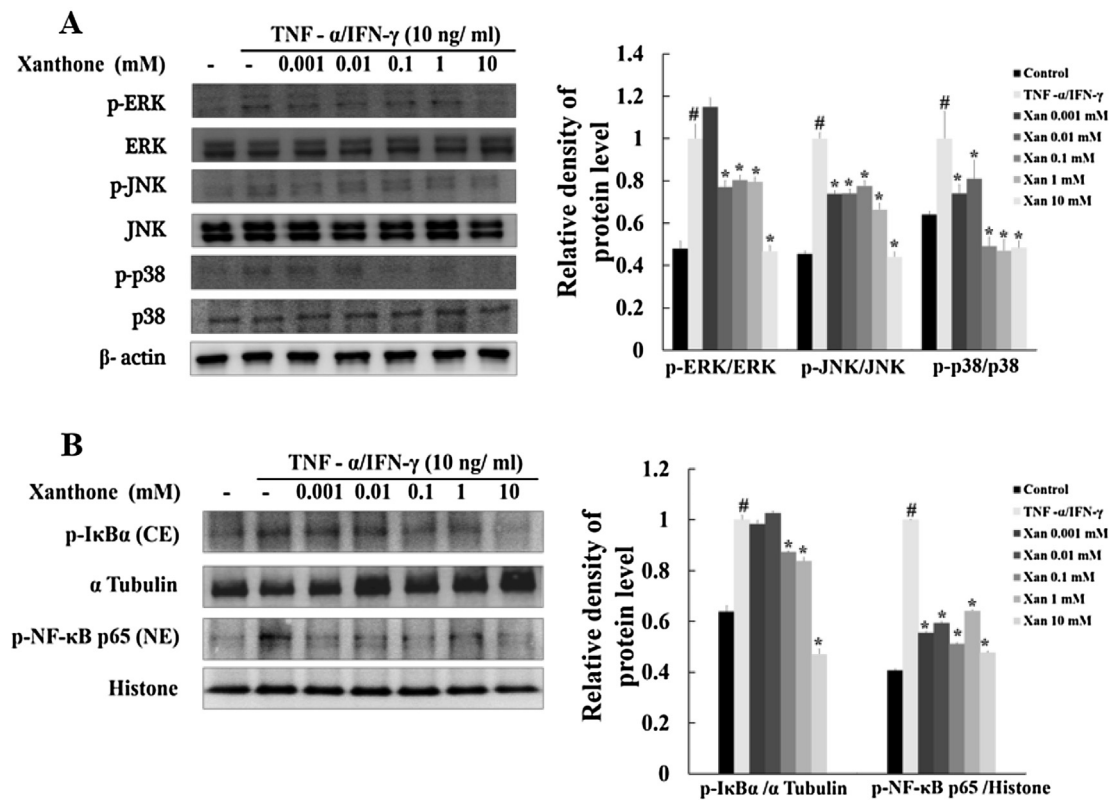


Fig. 2. Effects of xanthone on the phosphorylation of MAPKs and NF- κ B signaling pathway in TNF- α /IFN- γ -stimulated HaCaT cells. (A) The phosphorylation of ERK, JNK, and p38. (B) The phosphorylation of I κ B α and NF- κ B. CE, cytosol extracts; NE, nuclear extracts. The data represent the mean $\pm$ S.E.M. (# $p < 0.05$ vs. control group, * $p < 0.05$ vs. TNF- α /IFN- γ -stimulated group).

inflammatory diseases.

At present, ACD is commonly treated with anti-inflammatory drugs, such as corticosteroids, and immunosuppressants. However, the long-term use of corticosteroids results in tachyphylaxis and drug resistance [21]. Therefore, there is a need to identify new natural products that do not have considerable side effects. Xanthone is commonly found in a few higher plants, fungi, and lichens [22]. Xanthone has numerous pharmacological activities, including antioxidant, anticancer, and anti-inflammatory properties [23]. However, the molecular and cellular mechanisms underlying the suppressive effects of xanthone in ACD are yet to be clearly elucidated.

Therefore, this study aimed to evaluate the effects of xanthone on human keratinocytes and human mast cells *in vitro*, in addition to studying the effect in a murine model of ACD and anaphylactic shock reaction *in vivo*.

2. Materials and methods

2.1. Reagents

Xanthone is a heterocyclic compound with dibenzo- γ -pyrone as the main structure (Fig. 1A); ($C_{13}H_8O_2$, 97%), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), DNFB, compound 48/80, Evans blue and anti-dinitrophenyl (DNP)-IgE were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). DNP-BSA was purchased from Invitrogen (Gaithersburg, MD, USA). Roswell Park Memorial Institute (RPMI) 1640 medium, Iscove's Modified Dulbecco's Medium (IMDM), fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Gibco (Grand Island, NY, USA). Anti-phospho-I κ B α , NF- κ B (p65), histone, α -tubulin, JNK, p38, and β -actin antibodies were purchased from Santa Cruz Biotechnology (CA, USA). Anti-phospho-ERK, phospho-JNK, phospho-p38, and caspase-1

antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA, USA).

2.2. Cell culture

Human keratinocytes (HaCaT) were obtained from College of Veterinary Medicine, Chonbuk National University (Iksan, Republic of Korea) and the human mast cell line (HMC-1) were obtained from Sangji University (Wonju, Republic of Korea). The HaCaT cells were cultured in RPMI-1640 medium, HMC-1 cells were cultured in IMDM supplemented with 10% FBS, 100 units/mL penicillin, and 100 mg/mL streptomycin. The both cells were incubated at 37 °C in a humidified 5% CO₂ incubator.

2.3. Cell viability

The cells were seeded at 5×10^5 cells/well in a 24-well plate and stabilized overnight at 37 °C. The cells were treated with various concentrations of xanthone (0.001–100 mM) for 24 h. Subsequently, the MTT solution (5 mg/mL) was added to each well and incubated over 4 h. The crystallized formazan was lysed with DMSO and the colorimetric changes were measured at 540 nm using a Versamax microplate reader (Molecular Devices, CA, USA).

2.4. Enzyme-linked immunosorbent assay (ELISA)

The production of proinflammatory cytokines was measured by using a BD OptEIA ELISA kit, according to the manufacturer's instructions. Briefly, the 96-well microplates were pre-coated with capture antibodies and incubated overnight at 4 °C. After blocking 10% FBS in PBS for 1 h, the standards and samples were added and incubated for 2 h. The plates were then washed with 0.05% PBST, and the detection

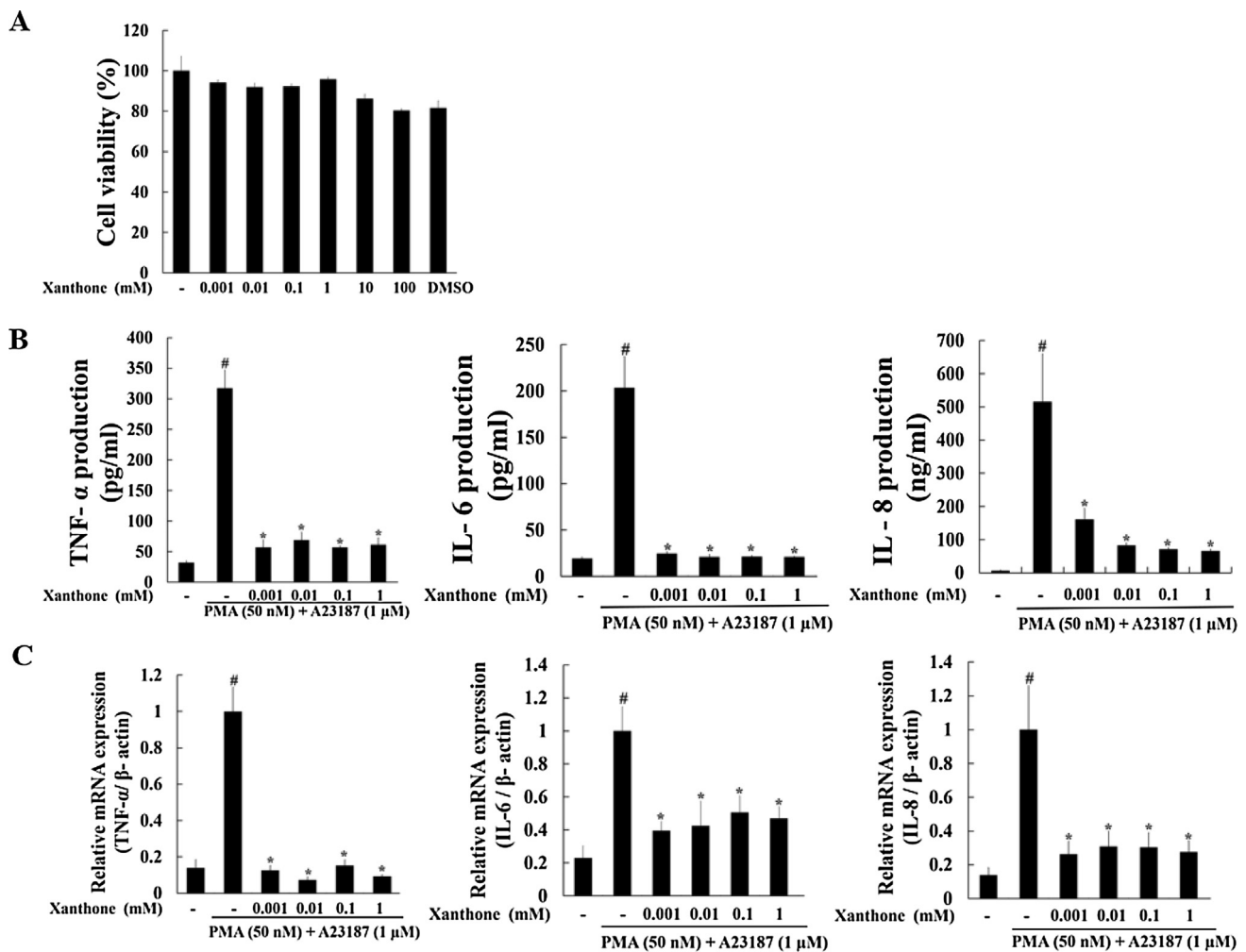


Fig. 3. Effects of xanthone on the production of pro-inflammatory mediators in PMACI-stimulated HMC-1 cells. (A) Cell viability. (B) The production levels of TNF- α , IL-6, and IL-8. (C) The mRNA expression of TNF- α , IL-6, and IL-8. The data represent the mean $\pm$ S.E.M. (# $p < 0.05$ vs. control group, * $p < 0.05$ vs. PMACI-stimulated group).

antibody/SAV-HPR enzyme solution was added to each well for 1 h. Subsequently, the plates were incubated with TMB substrate solution at room temperature for 30 min. The absorbance was measured at 450 nm using a microplate reader. The production of histamine was measured using an ELISA kit (BioVision, California, USA), according to the manufacturer's instructions.

2.5. RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

The total RNA was isolated from cells using an easy-BLUE RNA extract kit, (iNtRon Biotech, Seoul, Republic of Korea) according to the manufacturer's instructions. The total RNA was used as the template for first-strand complementary DNA (cDNA) synthesis from 1 μ g of RNA template and oligo dT, which were heated to 75 $^{\circ}$ C for 5 min. For cDNA synthesis, each sample was reverse transcribed for 60 min at 42 $^{\circ}$ C using a cDNA synthesis kit (BioFACT, Daejeon, Republic of Korea). PCR amplification was performed for 34 cycles, which comprised denaturation at 94 $^{\circ}$ C for 5 min and 30 s, annealing at 60 $^{\circ}$ C for 30 s, and extension at 72 $^{\circ}$ C for 45 s. The PCR products were separated by electrophoresis on a 1% agarose gel and visualized using B-BOXTM blue light LED epi-illuminator (SMOBIO Technology, Inc., Taiwan). The primer sequences are enlisted in Table 1. Real-time RT-PCR was performed using SYBR Green QPCR Master Mix, using an AriaMx Real-time PCR system (Agilent technologies, Santa Clara, USA). The primer sequences

are enlisted in Table 2.

2.6. Western blot analysis

For western blot analysis, the previously collected cells and dorsal skin tissues were rinsed with ice-cold PBS and lysed using PRO-PREP protein extraction solution (iNtRon Biotech, Seoul, Republic of Korea). The nuclear extracts were isolated using NEPER Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific, Rockford, IL, USA). The amount of protein was quantified using a Bio-Rad DC Protein Assay Kit (Bio-Rad, Hercules, CA, USA), mixed with a 2 $\times$ sample buffer, separated by 10% SDS polyacrylamide gel electrophoresis, and then transferred to PVDF membrane (Roche Diagnostics, Indianapolis, IL, USA). The membranes were incubated overnight with primary antibodies at 4 $^{\circ}$ C and horseradish peroxidase-conjugated secondary antibody (anti-mouse or anti-rabbit IgG) for 90 min. The protein bands were detected using an ECL western blotting luminal reagent (Santa Cruz) and recorded with a LAS-4000 Image Reader (Fujifilm Life Sciences, Tokyo, Japan).

2.7. Experimental animal model

Male BALB/c mice, aged 6 weeks, and ICR mice aged 4 weeks, were purchased from SAMTAKO (Osan, Republic of Korea). They were housed in a controlled environment at a temperature of 25 $^{\circ}$ C $\pm$ 2 $^{\circ}$ C,

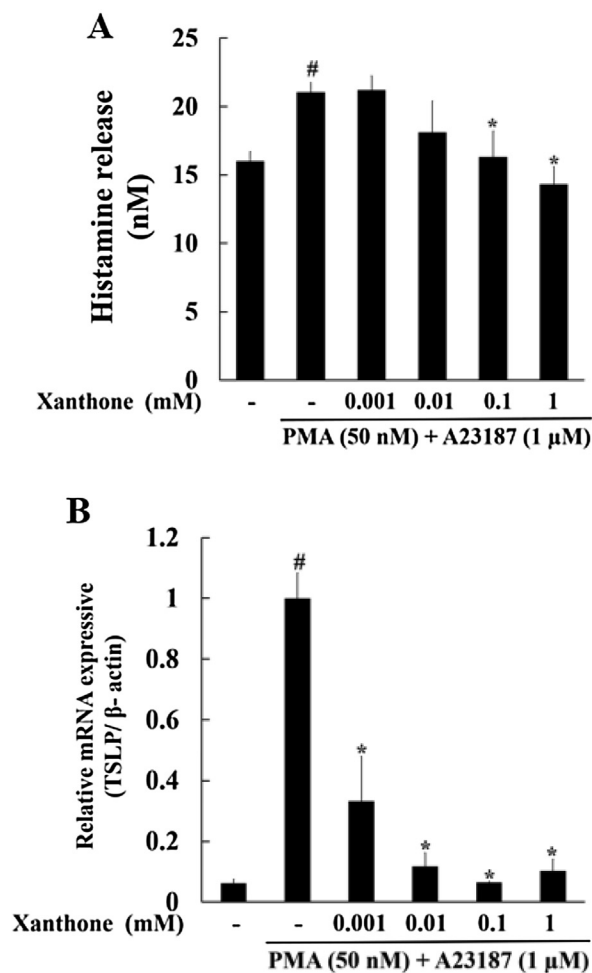


Fig. 4. Effects of xanthone on the production of allergic mediators in PMACI-stimulated HMC-1 cells. (A) The release of histamine. (B) The expression level of TSLP mRNA. The data represent the mean $\pm$ S.E.M. ([#] $p < 0.05$ vs. control group, ^{*} $p < 0.05$ vs. PMACI-stimulated group).

under a 12 h dark-light cycle. The animals were allowed access to diet and water ad libitum. All the mice were subjected to 7 days of purification prior to experimentation. All the animal experimental protocols were approved by the Committee on the Care of Laboratory Animal Resources, Chonbuk National University (CBNU 2019-065).

The procedure for the induction of ACD and treatment are described in [Supplementary Fig. S3](#). The BALB/c mice were divided into the following six groups ($n = 7$): group 1 served as the control, group 2 received 0.15% DNFB, group 3 received DNFB and 0.2 mg/kg xanthone, group 4 received DNFB and 2 mg/kg xanthone, group 5 received DNFB and 20 mg/kg xanthone and group 6 received DNFB and 1 mg/kg dexamethasone as positive control. The mice were sacrificed, then the serum and tissues of the dorsal skin were collected from each mouse.

2.8. Histological analyses

The dorsal skin tissues were collected and fixed in 10% formalin. The tissues were embedded in paraffin wax and sliced into 5 μ m thick sections. The deparaffinized skin sections were stained with hematoxylin and eosin (H&E). The mast cells in the skin were stained with toluidine blue (TB). The slices were mounted in permanent mounting medium (Vectra laboratories, Inc. Burlingame, CA, USA). All the stained tissue sections were then examined by light microscopy at 10 $\times$ magnification (Carl Zeiss Primo Star, USA).

2.9. Compound 48/80-induced anaphylactic shock

The ICR mice ($n = 6$) were intraperitoneally injected with PBS or compound 48/80 (8 mg/kg dissolved in PBS). Xanthone (0.2, 2, and 20 mg/kg) and dexamethasone (1 mg/kg) were orally administered 1 h prior to injecting compound 48/80. The survival rate was monitored for 75 min after the induction of anaphylactic shock.

2.10. PCA

Anti-DNP-IgE was intradermally injected at a dose of 50 ng per ear into the right ear of the ICR mice ($n = 6$) for 24 h. Xanthone (0.2, 2, and 20 mg/kg) and dexamethasone (1 mg/kg) were orally administered 1 h prior to the antigen challenge. DNP-BSA (100 μ g) dissolved in PBS containing 1% Evans blue, was intravenously injected into the tail vein of each mouse. One hour after the DNP-BSA injection, the mice were sacrificed, their right ears were removed and soaked in formaldehyde at 60 $^{\circ}$ C for 48 h; subsequently, the colorimetric changes were measured at 520 nm using a microplate reader.

2.11. Statistical analyses

All the results are represented as the mean $\pm$ S.E.M. The results were analyzed using Graph Pad Prism software, version 5.0 (Graph Pad Software, Inc, La Jolla, CA, USA). One-way analysis of variance with the Tukey post hoc test was used to identify the statistically significant differences. A value of $p < 0.05$ was considered significant.

3. Results

3.1. Effect of xanthone on the production of pro-inflammatory cytokines and chemokines in TNF- α /IFN- γ -stimulated HaCaT cells

Keratinocyte-derived inflammatory molecules amplify the skin immune responses associated with ACD and contribute to the disease process [24]. Treatment with xanthone at doses of 0.001 to 10 mM did not show cytotoxicity in HaCaT cells (Fig. 1B). The results demonstrated that stimulation of TNF- α /IFN- γ significantly increased the production of inflammatory mediators in the HaCaT cells compared with unstimulated cells. By contrast, treatment with xanthone doses of 0.001 to 10 mM significantly suppressed the production of IL-1 β , IL-6, and IL-8 (Fig. 1C) and the expression of IL-1 β , IL-6, and IL-8 mRNAs (Fig. 1D).

Chemokines are thought to play important roles during immune responses and inflammatory reactions by recruiting selected subsets of leukocytes to the target site [7]. Also, CCR4 ligands, such as TARC and MDC, play an important role in immune and inflammatory reaction. Those chemokines enhance the activation and recruitment of Th2 cell and production of Th2 related cytokines [8]. As shown in Fig. 1E, treatment with xanthone at doses of 0.001–10 mM significantly inhibited the TNF- α /IFN- γ -stimulated expression of TARC and MDC mRNAs in HaCaT cells. These results suggested that xanthone can modulate the expression of cytokines and Th2-associated chemokines, leading to the suppression of skin inflammation caused by the infiltration of inflammatory cells.

3.2. Effect of xanthone on the phosphorylation of MAPKs and NF- κ B in TNF- α /IFN- γ -stimulated HaCaT cells

MAPKs, NF- κ B, and the caspase-1 signaling pathway are activated in immune regulation, apoptosis, differentiation, and inflammation [18–20]. The results demonstrated that treatment with xanthone decreased the TNF- α /IFN- γ -stimulated phosphorylation of ERK, JNK, and p38 in HaCaT cells (Fig. 2A). Xanthone also inhibited the phosphorylation of I κ B α and NF- κ B (Fig. 2B). Additionally, treatment with xanthone significantly suppressed the activation of caspase-1 in HaCaT

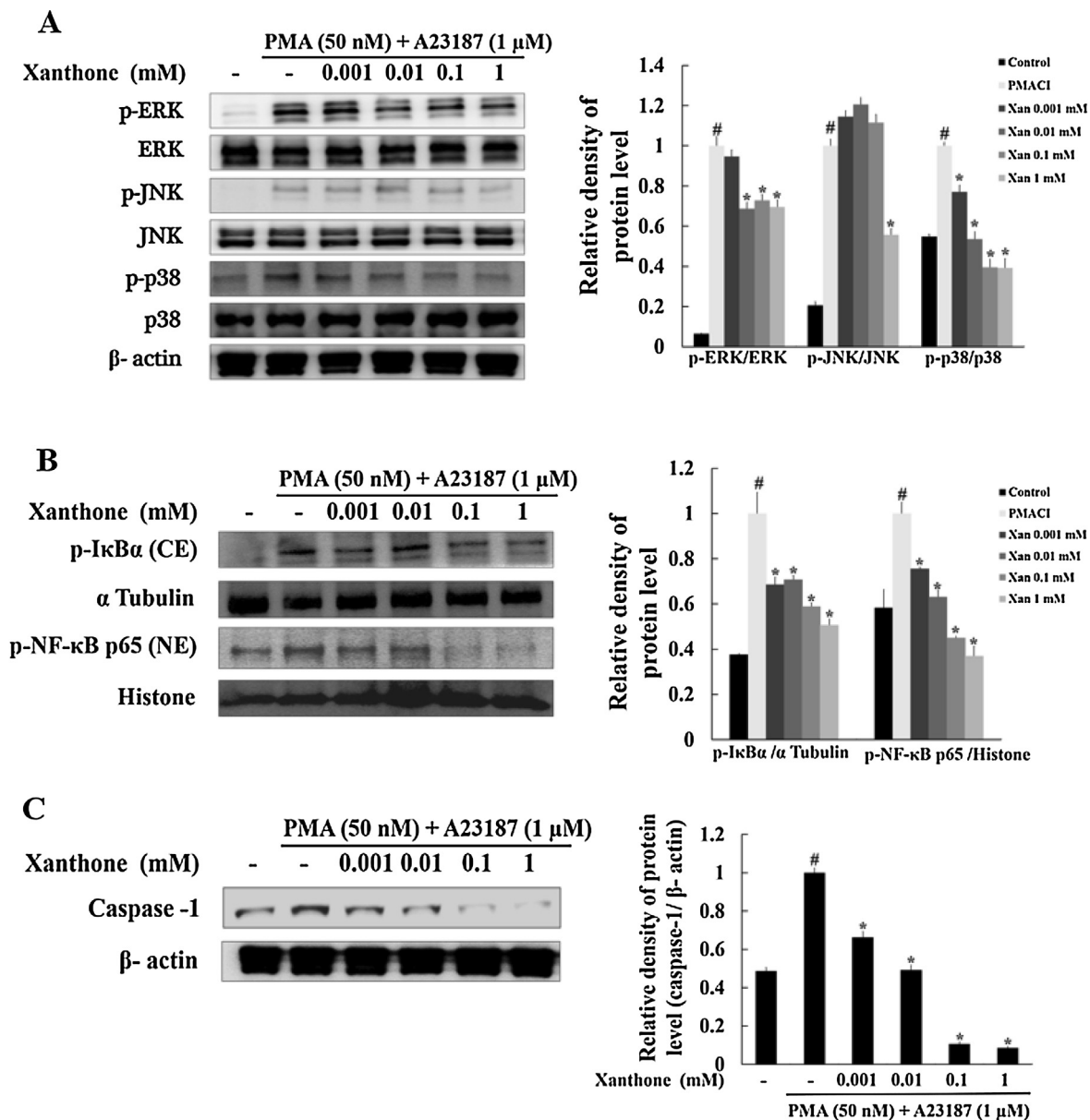


Fig. 5. Effects of xanthone on the phosphorylation of MAPKs, NF- κ B, and caspase-1 signaling pathway in PMACI-stimulated HMC-1 cells. (A) The phosphorylation of ERK, JNK, and p38. (B) The phosphorylation of I κ B α and NF- κ B (C) The activation of caspase-1. CE, cytosol extracts; NE, nuclear extracts. The data represent the mean $\pm$ S.E.M. (# p < 0.05 vs. control group, * p < 0.05 vs. PMACI-stimulated group).

cells (Supplementary Fig. S1). These results suggested that xanthone could regulate the inflammatory reaction by suppressing MAPKs and NF- κ B phosphorylation.

3.3. Effect of xanthone on the production of pro-inflammatory mediators in PMACI-stimulated HMC-1 cells

Mast cells play an important role in both adaptive and innate immunity and are increased in allergic inflammatory diseases [10]. As shown in Fig. 3A, treatment with xanthone at doses of 0.001 to 1 mM did not exert any cytotoxic effects in the HMC-1 cells. To determine whether xanthone suppressed the release of pro-inflammatory mediators in PMACI-stimulated HMC-1 cells, real-time RT-PCR and ELISA were used. The results revealed that stimulated with PMACI (50 nM PMA and 1 μ M A23187) significantly increased the production of inflammatory mediators in HMC-1 cells, compared to that in the unstimulated cells. However, the production of cytokines TNF- α , IL-6, and chemokine IL-8 (Fig. 3B), and the expression of their mRNAs (Fig. 3C),

were suppressed by xanthone treatment at doses of 0.001 to 1 mM. These findings collectively suggested that xanthone effectively suppressed the production of inflammatory mediators in PMACI-stimulated mast cells.

3.4. Effect of xanthone on the production of allergic mediators in PMACI-stimulated HMC-1 cells

To determine whether xanthone suppressed the release of allergic mediators (histamine and TSLP) from the PMACI-stimulated mast cells, ELISA and RT-PCR were used. As shown in Fig. 4A, the release of histamine in HMC-1 cells were markedly increased by PMACI stimulation compared with unstimulated cells. By contrast, treatment with xanthone at doses of 0.001–1 mM decreased the release of histamine. As shown in Supplementary Fig. S2, TSLP expression was effectively induced by PMACI treatment for 45 min. The various concentration of xanthone 0.001 to 1 mM significantly reduced the expression of TSLP mRNA in PMACI-stimulated HMC-1 cells (Fig. 4B). The results

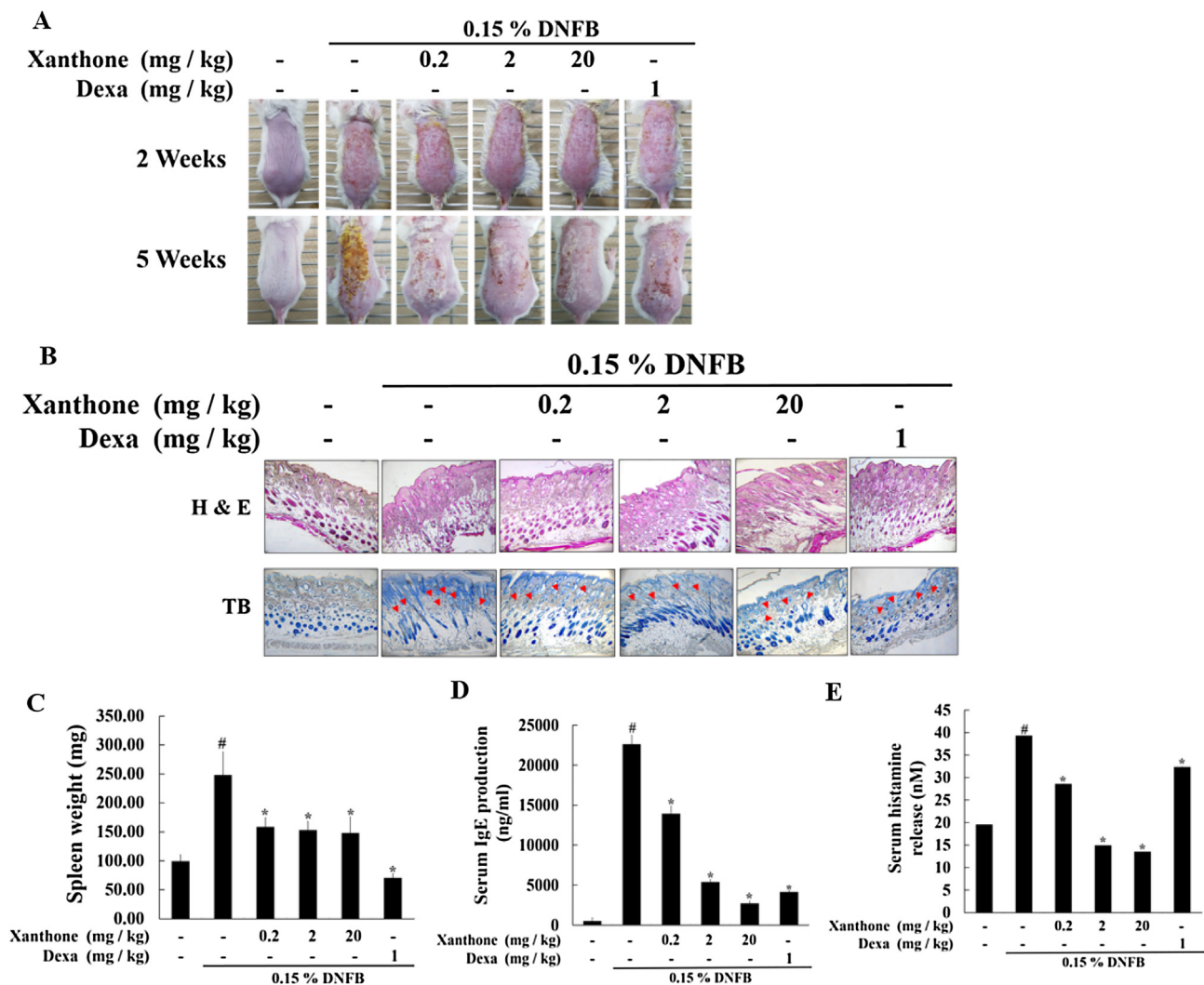


Fig. 6. Effect of xanthone on DNFB-induced dermatitis in mice. (A) Comparison of the DNFB-induced dermatitis in BALB/c mice. (B) H&E and TB staining. The arrows indicate the TB-stained mast cells in the dermis (10 × magnification). (C) The weight of the spleen. (D) The serum levels of IgE. (E) The serum levels of histamine. The data represent the mean ± S.E.M. of the changes in the observations ([#] $p < 0.05$ vs. control group, ^{*} $p < 0.05$ vs. DNFB-induced group).

suggested that xanthone treatment decreased the production of allergic mediators in mast cells.

3.5. Effect of xanthone on the phosphorylation of MAPKs, NF- κ B and caspase-1 in PMACI-stimulated HMC-1 cells

To explore whether xanthone inhibited the allergic inflammatory responses, the phosphorylation of MAPKs, NF- κ B, and caspase-1 signaling pathway were detected by western blot analysis. As shown in Fig. 5A, B and C, PMACI significantly induced the phosphorylation of MAPKs and NF- κ B and activation of caspase-1 in HMC-1 cells. Treatment with xanthone suppressed the PMACI-stimulated phosphorylation of ERK, p38 and JNK (Fig. 5A). In addition, xanthone markedly reduced the phosphorylation of I κ B α and NF- κ B in PMACI-stimulated HMC-1 cells (Fig. 5B). Furthermore, the activation of caspase-1 was inhibited by the treatment with xanthone in PMACI-stimulated HMC-1 cells (Fig. 5C).

3.6. Effect of xanthone on DNFB-induced dermatitis in mice

Induced dermatitis is characterized by a potent skin inflammation associated with frequent scratching behavior [25]. To evaluate the regulatory effects of xanthone in induced dermatitis, BALB/c mice with

DNFB-induced dermatitis was used as a model for ACD. The treatment with xanthone, at doses of 0.2, 2, and 20 mg/kg significantly reduced the DNFB-induced skin lesion (Fig. 6A). H&E staining showed that the epidermal thickness and the number of TB-stained mast cells were significantly higher in the skin lesions of the DNFB group, in comparison with those of the normal group. By contrast, treatment with xanthone significantly reduced the DNFB-induced hyperplasia and the population of inflammatory mast cells (Fig. 6B) and weights of the spleen in the xanthone-treated groups, which were significantly lower than that of the DNFB group (Fig. 6C). The serum levels of IgE, histamine, and IL-6 were markedly reduced following xanthone administration, in comparison with those of the DNFB group (Fig. 6D, E, and Supplementary Fig. S4). These results demonstrated that xanthone reduced epidermal hyperplasia by inhibiting the production of pro-inflammatory mediators.

3.7. Effect of xanthone on the production of pro-inflammatory cytokines and phosphorylation of MAPKs in skin lesions of DNFB-induced dermatitis mice

To evaluate the effects of xanthone on the production of pro-inflammatory cytokines in the dorsal skin tissues of the mice with DNFB-induced dermatitis, the levels of pro-inflammatory cytokines were

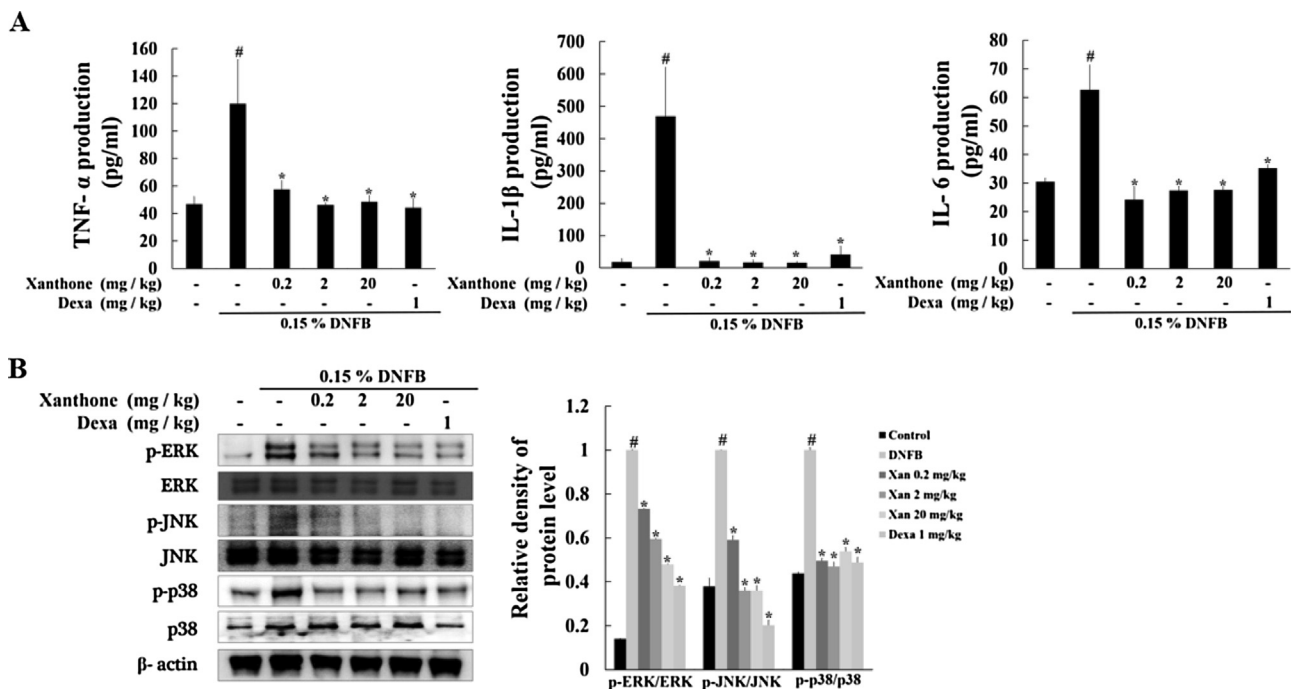


Fig. 7. Effects of xanthone on the production of pro-inflammatory mediators and phosphorylation of MAPKs in DNFB-induced dermatitis. (A) The production levels of TNF- α , IL-1 β , and IL-6 in the skin tissues. (B) The phosphorylation of ERK, JNK, and p38. The data represent the mean $\pm$ S.E.M. of the changes in the observations ($\# p < 0.05$ vs. control group, $*p < 0.05$ vs. DNFB-induced group).

analyzed using ELISA. As shown in Fig. 7A, DNFB challenge increased the levels of TNF- α , IL-1 β , and IL-6 in the dorsal skin lesions, whereas treatment with xanthone significantly reduced the levels of TNF- α , IL-1 β , and IL-6, as observed in the xanthone-treated groups. Additionally, the administration of xanthone significantly suppressed the phosphorylation of ERK, JNK, and p38 in the dorsal skin, in comparison with that in the DNFB-induced group (Fig. 7B). These results indicated that treatment with xanthone suppressed the immune response by regulating the phosphorylation of the MAPKs pathway.

3.8. Effect of xanthone on the anaphylactic shock and PCA reaction in mice

Anaphylactic shock induced by compound 48/80 is the standard *in vivo* model for studying allergic reactions [26]. As shown in Fig. 8A, treatment with xanthone (2 mg/kg) increased the survival rate by 66.7%, also treatment with xanthone (20 mg/kg) significantly increased the survival rate by 83.3% after the induction of anaphylactic shock. PCA is the most widely used murine model for studying immediate allergic reactions [27]. Interestingly, the administration of xanthone at a dose of 20 mg/kg visibly suppressed the IgE-mediated PCA reaction in mice in comparison with that observed after dexamethasone treatment (Fig. 8B).

4. Discussion

ACD is a T cell-mediated hypersensitivity and inflammatory skin reactions occurring in sensitized individuals at the site of contact allergens challenge [3,4]. At present, most topical therapies for ACD include corticosteroids and non-steroidal anti-inflammatory drugs [21]. However, these agents are often associated with severe adverse effects. Therefore, there is a need to develop novel and effective therapies for the treatment of ACD. Xanthone has a wide range of pharmacological properties, enabling its use in the treatment of numerous diseases [23]. Numerous studies have demonstrated that haptens directly activate the inflammatory response of keratinocytes during sensitization, resulting in the expression of inflammatory molecules [4,13]. The results of this study demonstrated that treatment with xanthone significantly

suppressed the production of pro-inflammatory cytokines and chemokines in TNF- α /IFN- γ -stimulated HaCaT cells.

Contact allergens trigger the mast cells, inducing histamine secretion, which aids degranulation and is a prominent contributor to allergic diseases during the sensitization stage of ACD [9,11]. The allergy-related inflammation mediators, including TSLP, MAPKs and NF- κ B, represent the severity of allergic inflammation in chronic allergic conditions [28]. Also, NF- κ B activation affects the TSLP expression, which sustains Th2 related allergic inflammation [29]. The results of this study revealed that xanthone inhibited the production of TNF- α , IL-6, IL-8, and the release of histamine, and the expression of TSLP in PMACI-stimulated HMC-1 cells. These data collectively suggested that xanthone could be used to treat related allergic diseases. Both MAPKs activation and the translocation of NF- κ B to the nucleus, where it participates in the expression of many pro-inflammatory genes [18–20]. Additionally, caspase-1 activation is necessary for the NF- κ B signaling pathway [20]. In this study, treatment with xanthone significantly inhibited the phosphorylation of MAPKs, the nuclear translocation of NF- κ B, and the caspase-1 pathway in the keratinocytes and mast cells. When the skin of a mouse is repeatedly exposed to DNFB, it exhibits epidermal thickness [4,5]. Treatment with xanthone improved the severity of the skin lesions in mice with DNFB-induced dermatitis.

The secretion of cytokines is an important contributor to the progression of ACD because it enhances the production of acute phase proteins [12–16]. In the present study, the administration of xanthone decreased the serum levels of IgE, histamine, and pro-inflammatory cytokines by inhibiting MAPKs in DNFB-induced mice. These results indicated that the administration of xanthone suppressed the immune response by regulating the MAPKs signaling pathway; therefore, it could be used to treat ACD. Moreover, numerous studies have demonstrated that mast cell degranulation can also be elicited by the synthetic compound 48/80 or IgE and is directly used to study the mechanism of anaphylaxis owing to the convenience of the protocol [26,27]. In this study, the administration of xanthone significantly inhibited the allergic responses by suppressing the anaphylactic reaction in mice.

In conclusion, this study demonstrated that treatment with xanthone effectively suppressed the production of pro-inflammatory

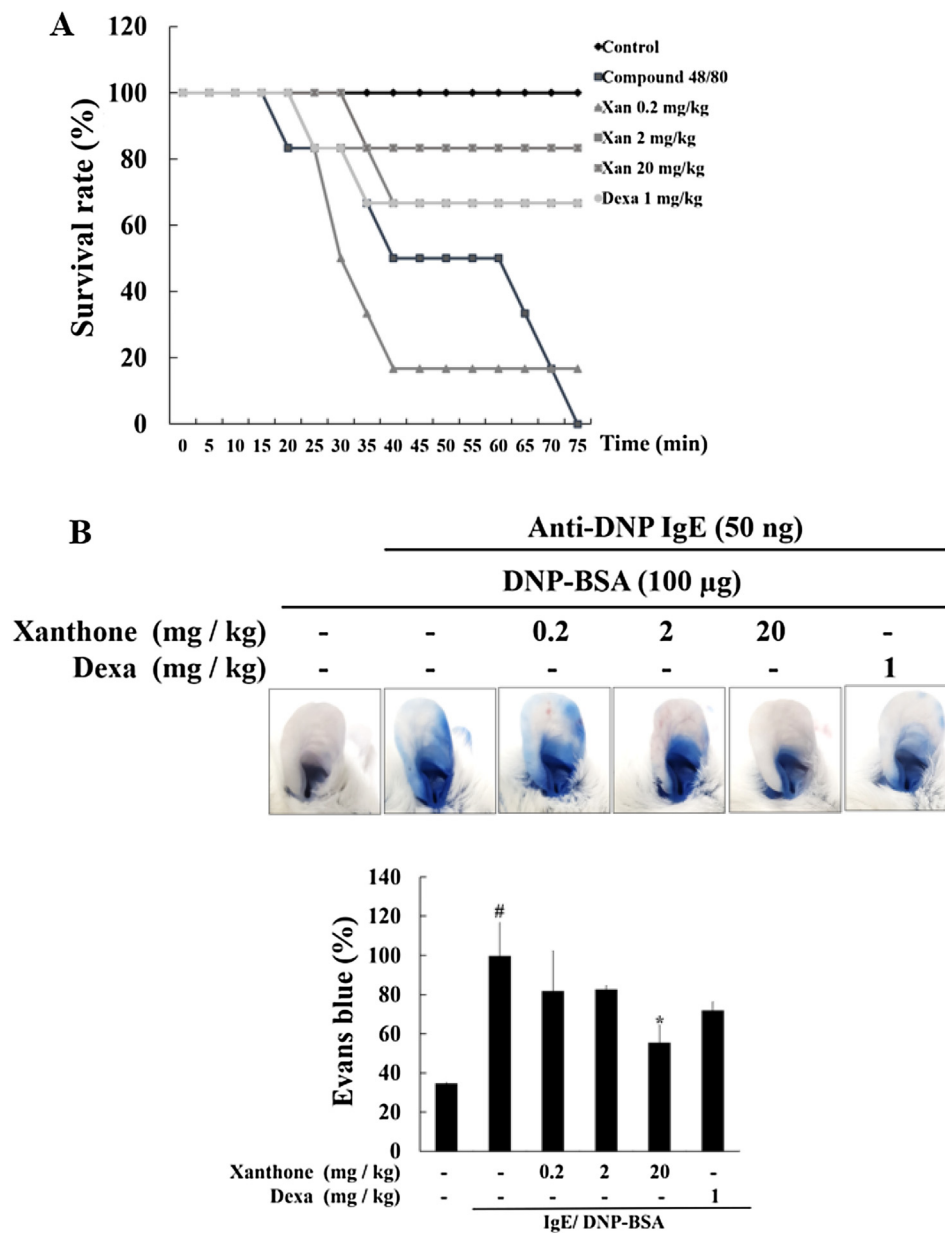


Fig. 8. Effects of xanthone on compound 48/80-induced anaphylactic shock and IgE-mediated PCA reaction. (A) The survival rates of the mice were monitored for 75 min. (B) Images of extravasated Evans blue dye from mice ears after PCA reaction. The data represent the mean $\pm$ S.E.M. (# $p < 0.05$ vs. control group, * $p < 0.05$ vs. IgE-mediated group).

mediators in the keratinocytes and mast cells *in vitro*. Additionally, xanthone suppressed the immune response by regulating MAPKs, NF- κ B, and caspase-1 pathway in keratinocytes and mast cells. Furthermore, the administration of xanthone exhibited anti-inflammatory and anti-allergic activities, which were evidenced by the suppression of the symptoms of DNFB-induced ACD, the systemic anaphylaxis induced by compound 48/80, and the PCA reaction mediated by IgE in murine model. Thus, the results suggest that xanthone could be a potential therapeutic agent for allergic inflammatory diseases such as dermatitis and anaphylaxis.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2019.106061>.

References

- [1] L. Fonacier, I. Noor, Contact dermatitis and patch testing for the allergist, *Ann. Allergy Asthma Immunol.* 120 (2018) 592–598.
- [2] S.J. Galli, M. Tsai, A.M. Piliponsky, The development of allergic inflammation, *Nature*. 454 (2008) 445–454.
- [3] S. Sebastiani, C. Albanesi, O. De Pita, P. Puddu, A. Cavani, G. Girolomoni, The role of chemokines in allergic contact dermatitis, *Arch. Dermatol. Res.* 293 (2002) 552–559.
- [4] P. Saint-Mezard, M. Krasteva, C. Chavagnac, S. Bosset, H. Akiba, J. Kehren, J. Kanitakis, D. Kaiserlian, J.F. Nicolas, F. Berard, Afferent and efferent phases of allergic contact dermatitis (ACD) can be induced after a single skin contact with

- haptens: evidence using a mouse model of primary ACD, *J. Invest. Dermatol.* 120 (2003) 641–647.
- [5] A.V. Gorbachev, R.L. Fairchild, CD4⁺CD25⁺ regulatory T cells utilize FasL as a mechanism to restrict DC priming functions in cutaneous immune responses, *Eur. J. Immunol.* 40 (2010) 2006–2015.
 - [6] A. Grone, Keratinocytes and cytokines, *Vet. Immunol. Immunopathol.* 88 (2002) 1–12.
 - [7] Y. Shimada, K. Takehara, S. Sato, Both Th2 and Th1 chemokines (TARC/ CCL17, MDC/CCL22, and Mig/CXCL9) are elevated in sera from patients with atopic dermatitis, *J. Dermatol. Sci.* 34 (2004) 201–208.
 - [8] L.Y. Al-Ayadhi, G.A. Mostafa, Elevated serum levels of macrophage-derived chemokine and thymus and activation-regulated chemokine in autistic children, *J. Neuroinflammation.* 10 (2013) 1–7.
 - [9] S.J. Galli, M. Tsai, Mast cells in allergy and infection: versatile effector and regulatory cells in innate and adaptive immunity, *Eur. J. Immunol.* 40 (2010) 1843–1851.
 - [10] R.L. Thurmond, E.W. Gelfand, P.J. Dunford, The role of histamine H₁ and H₄ receptors in allergic inflammation: the search for new antihistamines, *Nat. Rev. Drug Discov.* 7 (2008) 41–53.
 - [11] E.B. Thangam, E.A. Jemima, H. Singh, M.S. Baig, M. Khan, C.B. Mathias, M.K. Church, R. Saluja, The role of histamine and histamine receptors in mast cell-mediated allergy and inflammation: the hunt for new therapeutic targets, *Front. Immunol.* 9 (2018) 1–9.
 - [12] Y.J. Liu, Thymic stromal lymphopoietin and OX40 ligand pathway in the initiation of dendritic cell-mediated allergic inflammation, *J. Allergy. Clin. Immunol.* 120 (2007) 238–244.
 - [13] M.D. Turner, B. Nedjai, T. Hurst, D.J. Pennington, Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease, *Biochim. Biophys. Acta. Mol. Cell Res.* 1843 (2014) 2563–2582.
 - [14] S. Nakae, H. Suto, M. Kakurai, J.D. Sedgwick, M. Tsai, S.J. Galli, Mast cells enhance T cell activation: importance of mast cell-derived TNF, *Proc. Natl. Acad. Sci. USA* 102 (2005) 6467–6472.
 - [15] R.T. Boudreau, D.W. Hoskin, T.J. Lin, Phosphatase inhibition potentiates IL-6 production by mast cells in response to Fcεpsilon RI-mediated activation: involvement of p38 MAPK, *J. Leukoc. Biol.* 76 (2004) 1075–1081.
 - [16] F. Martinon, K. Burns, J. Tschopp, The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of pro IL-beta, *Mol. Cell.* 10 (2002) 417–426.
 - [17] B. Houshmand, M. Hajilooi, A. Rafiei, M. Bidgoli, S. Soheilifar, Evaluation of IL-8 gene polymorphisms in patients with periodontitis in Hamedan, Iran, *Dent. Res. J. (Isfahan)* 9 (2012) 427–432.
 - [18] G.L. Johnson, R. Lapadat, Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases, *Science.* 298 (2002) 1911–1912.
 - [19] P.A. Baeuerle, D. Baltimore, I kappa B: a specific inhibitor of the NF-kappa B transcription factor, *Science.* 242 (1988) 540–546.
 - [20] M. Lamkanfi, M. Kalai, X. Saelens, W. Declercq, P. Vandenabeele, Caspase-1 activates nuclear factor of the kappa-enhancer in B cells independently of its enzymatic activity, *J. Biol. Chem.* 279 (2004) 24785–24793.
 - [21] D.R.D.F. James, F.F. Sheila, Corticosteroids: options in the era of steroid- sparing therapy, *J. Am. Acad. Dermatol.* 53 (2005) 50–S58.
 - [22] K.S. Masters, S. Brase, Xanthones from fungi, lichens, and bacteria: the natural products and their synthesis, *Chem. Rev.* 112 (2012) 3717–3776.
 - [23] I. Shagufta, Ahmad, Recent insight into the biological activities of synthetic xanthone derivatives, *Eur. J. Med. Chem.* 116 (2016) 267–280.
 - [24] C. Albanesi, Keratinocytes in allergic skin diseases, *Curr. Opin. Allergy. Clin. Immunol.* 10 (2010) 452–456.
 - [25] S.C. Han, G.J. Kang, Y.J. Ko, H.K. Kang, S.W. Moon, Y.S. Ann, E.S. Yoo, Fermented fish oil suppresses T helper 1/2 cell response in a mouse model of atopic dermatitis via generation of CD4⁺ CD25⁺ Foxp3⁺ T cells, *BMC. Immunol.* 13 (2012) 1–12.
 - [26] M. Schemann, E.M. Kugler, S. Buhner, C. Eastwood, J. Donovan, W. Jiang, D. Grundy, The mast cell degranulator compound 48/80 directly activates neurons, *PLoS One.* 7 (2012) 1–9.
 - [27] F.D. Finkelman, Anaphylaxis: Lessons from mouse models, *J. Allergy Clin. Immunol.* 120 (2007) 506–515.
 - [28] H.J. Yang, M.J. Kang, S.N. Kang, N.R. Moon, D.S. Kim, N.R. Lee, K.S. Kim, S.M. Park, Topical treatments of Saussurea costus root and Thuja orientalis L. synergistically alleviate atopic dermatitis-like skin lesions by inhibiting protease-activated receptor-2 and NF-κB signaling in HaCaT cells and Nc/Nga mice, *J. Ethnopharmacol.* 199 (2017) 97–105.
 - [29] T. Bieber, Atopic dermatitis, *Ann. Dermatol.* 22 (2010) 125–137.

Supplementary figures

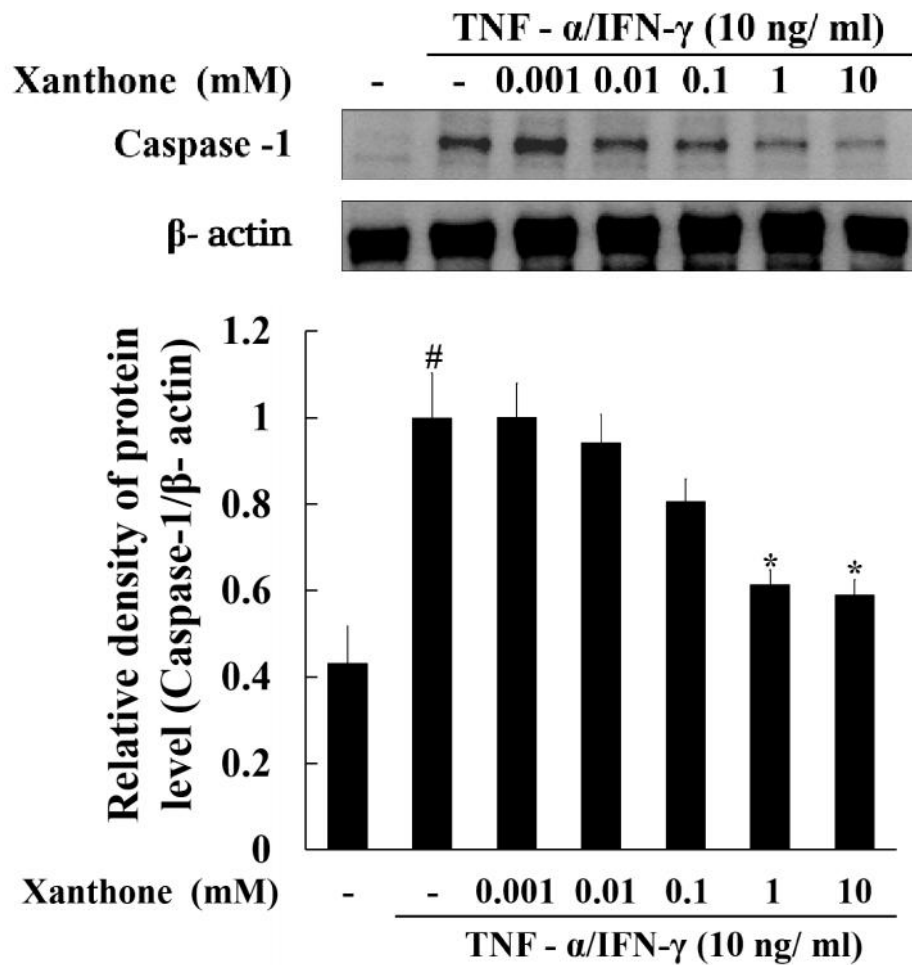


Fig. S1. Effect of xanthone on the activation of caspase-1 in TNF- α /IFN- γ -stimulated HaCaT cells. The activation of caspase-1 was measured by western blot analysis. The data represent the mean $\pm$ SEM (# $p < 0.05$ vs. control group, * $p < 0.05$ vs. TNF- α /IFN- γ -stimulated group).

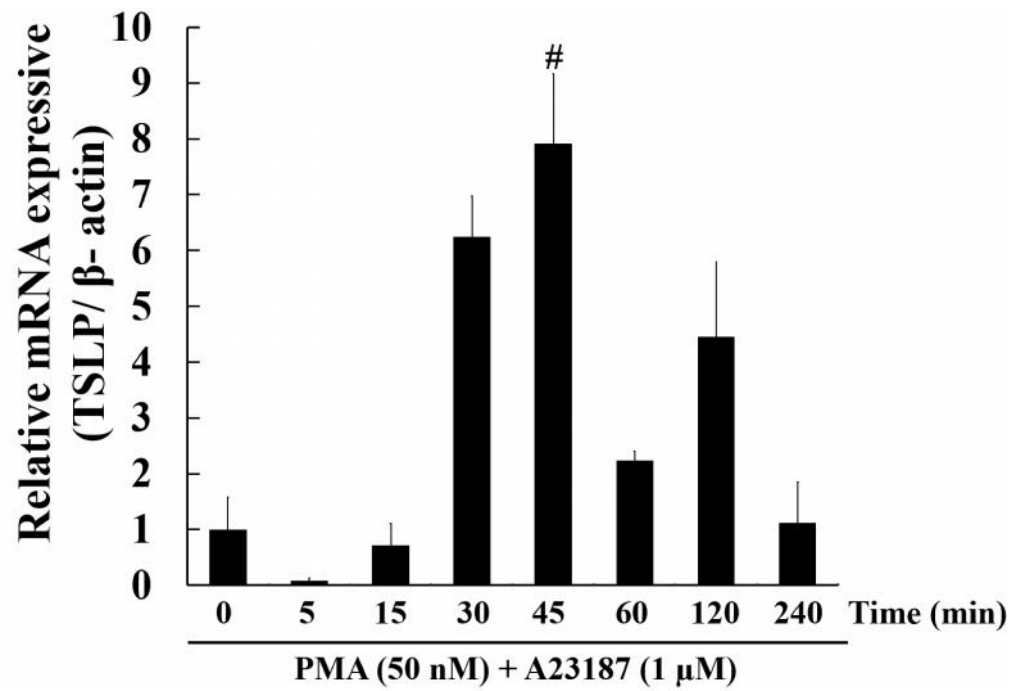


Fig. S2. Stimulatory effect of PMACI on the expression of TSLP in HMC-1 cells. HMC-1 cells (3×10^6 cells/well) were treated with PMACI (50 nM PMA and 1 μ M A23187) for 0, 5, 15, 30, 45, 60, 120, and 240 min. The expression of TSLP mRNA was determined by real-time RT-PCR. The data represent the mean $\pm$ SEM (# $p < 0.05$ vs. 0 min).

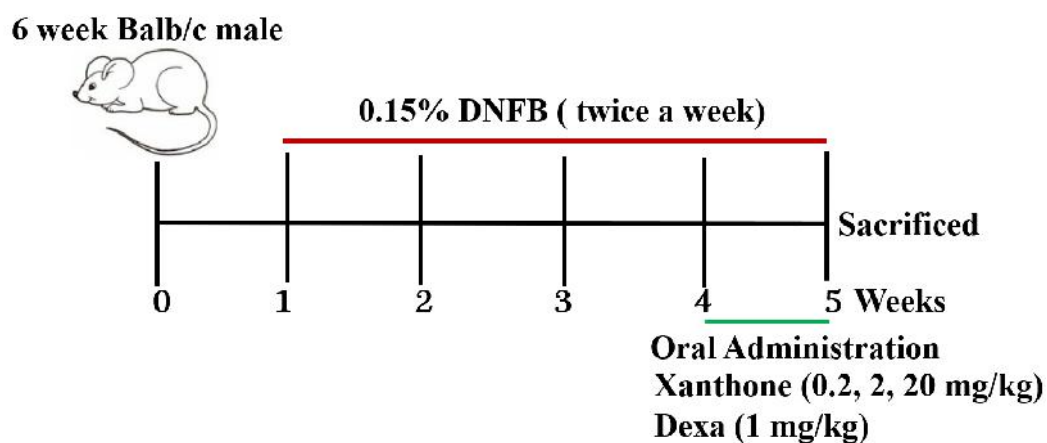


Fig. S3. Effect of xanthone on DNFB-induced dermatitis in mice. Schematic diagram of the study protocol. BALB/c mice ($n = 7$) were sensitized by the application of 100 μ L of 0.15 % DNFB in an acetone-olive oil combination (3: 1) or a vehicle (acetone/olive oil = 3: 1) on the dorsal skin of the mice, twice a week, for a total of 5 weeks. Xanthone, at doses of 0.2, 2, and 20 mg/kg, and dexamethasone, at a dose of 1 mg/kg, were orally administered to the mice with DNFB-induced dermatitis for a period of 1 week.

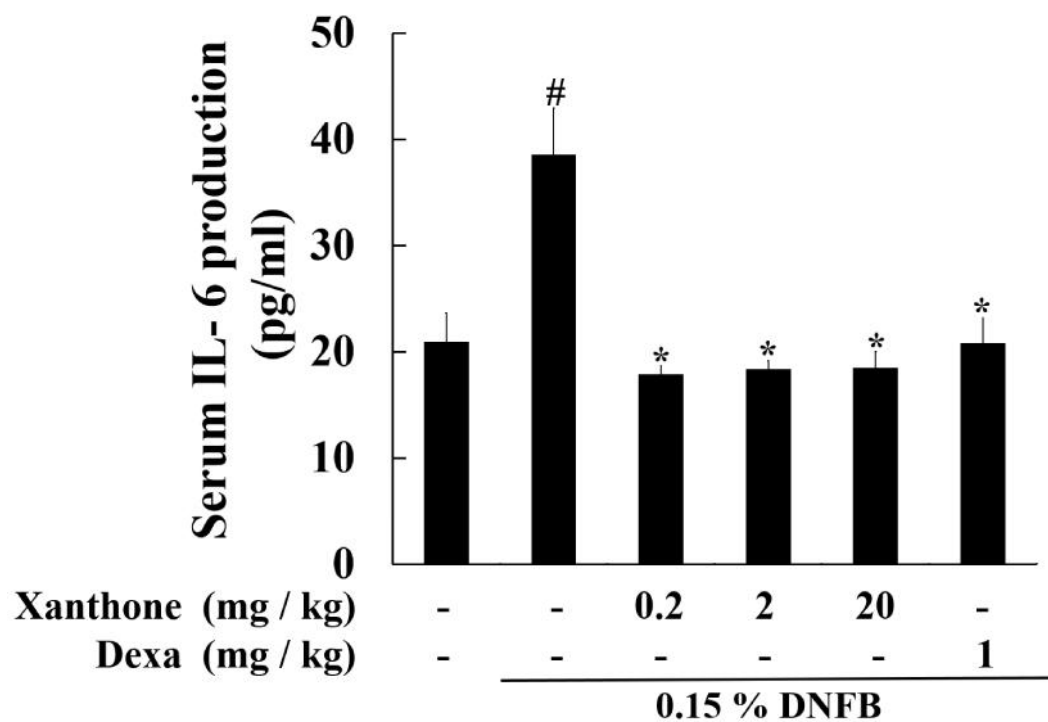


Fig. S4. Effect of xanthone on the serum level of IL-6 in DNFB-induced dermatitis. The serum level of IL-6 was measured by ELISA. The data represent the mean $\pm$ SEM of the changes in the observations (# $p < 0.05$ vs. control group, * $p < 0.05$ vs. DNFB-induced group).