Therapeutic effects of quinine in a mouse model of atopic dermatitis

QIAN ZHANG[1-3], HONGJING JIANG[3,4], MIAO LIU[5], XINCHEN LI[5], MURONG ZHOU[2,6], YANSI LYU[1], JINGKAI HUANG[1], SI CHEN[3,7] and LI WANG[1]

[1]Department of Dermatology, Shenzhen University General Hospital, Shenzhen University;
[2]College of Physics and Optoelectronic Engineering, Shenzhen University; [3]Shenzhen University Health Science Center;
[4]Department of Microbiology, Shenzhen University Health Science Center, Shenzhen, Guangdong 518060;
[5]School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xian, Shaanxi 710021;
[6]Guangdong and Hong Kong Joint Research Center for Optical Fiber Sensors, Shenzhen University;
[7]Department of Immunology, Shenzhen University Health Science Center, Shenzhen, Guangdong 518060, P.R. China

Abstract. Atopic dermatitis (AD) is a chronic inflammatory skin disease that seriously affects quality of life. Quinine is a bitter taste receptor agonist that exhibits antimalarial effects. The aim of the present study was to examine the therapeutic effects of quinine in AD-like mice. AD was induced with 2,4-dinitrochlorobenzene, and the mice were treated with 10 mg/kg quinine for 1, 4 and 7 days. A total of 60 BALB/c mice were divided into the following groups: Healthy, AD-like, AD-like + quinine and healthy + quinine, with 1, 4 and 7 days groups for each treatment. Blood was extracted from all mice and ELISA was performed to detect immunoglobulin E (IgE) levels. H&E-stained tissue sections were prepared from skin lesions on the backs of the mice and pathological changes were observed. Cytokines were detected via ELISA, and the filaggrin (FLG) and kallikrein-7 (KLK7) proteins were detected via western blotting and immunohistochemistry. IKKα and NF-κB mRNA were analyzed via reverse transcription-quantitative PCR. Quinine ameliorated skin damage in the AD-like mice, reduced IgE expression in the blood, inhibited expression of IKKα and NF-κB, reduced cytokine secretion, reduced KLK7 expression, reduced scratching frequency, increased FLG expression and repaired the skin barrier. These results suggested that quinine exhibited therapeutic effects in AD-like mice.

Introduction

Atopic dermatitis (AD, also known as atopic eczema) is a chronic inflammatory skin disease that affects 2-20% of the general population, and is the most significant non-fatal health burden caused by skin disease (1,2). AD results in major social and psychological burdens for patients and their relatives, impacting the social functioning and psychological well-being of patients (3). Current approaches for preventing and treating AD focus on antibiotics, corticosteroids, immunomodulators, immunosuppressants, skincare and lifestyle changes; however, the effects of current treatments are not ideal, and an optimal AD treatment needs to be developed to improve patient prognosis, and reduce the burden on patients and their families (4-6). AD is typically associated with type I allergic diseases, including allergic rhinitis and asthma (7). A major marker of the disease is elevated serum total immunoglobulin E (IgE) levels, which are observed in ~80% of patients with AD (8). In fact, patients with AD develop IgE antibody responses to a variety of environmental allergens and autoantigens (3).

It has been proposed that bitter taste receptors (TAS2Rs) (9) only exist on the tongue, and that their activation enables the perception of bitterness (10). However, a previous study has found that TAS2Rs are also present in the respiratory system (11). Children with severe asthma exhibit increased TAS2R expression in leukocytes, which are associated with mast cells (12). Administration of a TAS2R agonist can inhibit IgE-dependent mast cell activation, thereby reducing IgE expression (13).

Quinine is a TAS2R agonist that was purified in 1820 (14). A large number of medical cases have reported that quinine is a drug specific for malarial fever (15,16). However, quinine has been reported to treat or alleviate numerous nonmalarial diseases, including ulcers, hemorrhoids and gastric

Correspondence to: Dr Si Chen, Department of Immunology, Shenzhen University Health Science Center, A7 Building, 1066 Xueyuan Avenue, Nanshan, Shenzhen, Guangdong 518060, P.R. China
E-mail: chensi@szu.edu.cn

Professor Li Wang, Department of Dermatology, Shenzhen University General Hospital, Shenzhen University, Outpatient Building, 1098 Xueyuan Avenue, Nanshan, Shenzhen, Guangdong 518060, P.R. China
E-mail: pfkwangli@126.com

inflammation (17). A TAS2R agonist was found to reduce IgE levels in mice with allergic asthma and to reduce clinical symptoms (18). Shaw *et al* (19) also detected TAS2R expression in human skin. In the present study, quinine was used to treat AD-like mice. ELISAs, western blotting, immunohistochemistry and reverse transcription-quantitative PCR (RT-qPCR) were used to investigate the effect and mechanism of quinine in the treatment of AD.

Materials and methods

Reagents and drugs. The quinine (purity, >99%) employed in the present study was purchased from MedChemExpress (cat. no. HY-D0143), the 2,4-dinitrochlorobenzene (DNCB) was supplied by Sigma-Aldrich; Merck KGaA (cat. no. 237329). TRIzol® reagent was purchased from Invitrogen; Thermo Fisher Scientific, Inc.. The reverse transcription kit (PrimeScript™ RT-PCR kit) and the SYBR-Green kit (both Takara Bio, Inc.) were employed for RT-qPCR analysis.

Induction of the atopic dermatitis-like mice and treatment of quinine in mice. A total of 60 male BALB/c mice (age, 5-6 weeks; weight, 17-20 g; n=5/group) were purchased from the Guangdong Province Medical Experimental Center. AD-like skin lesions in the mice were induced as previously described (20,21). The experimental schedule is presented in Fig. 1A. Specifically, the procedure was as follows: Before the experiment, the back hair above the hind legs was shaved over an area of 2.5x2.5 cm. On days 1 and 2, the mice were coated with a 100-μl mixture of 0.5% DNCB and acetone/olive oil (3:1) on the shaved areas. From day 3 to 6, nothing was applied. On day 7, and then every 2 days afterwards until day 28, the mice were coated with 100 μl of the above mixture to induce an AD-like phenotype (Fig. 1B). The skin severity was evaluated based on four symptoms (erythema/hemorrhage, edema, excoriation/erosion and dryness) and defined as a sum of the individual scores (0, no symptoms; 1, mild; 2, moderate; 3, severe) (22).

In a control group of mice, the acetone/olive oil mixture was applied to the back area with the same time and quantity of application as the model group (Fig. 1C). All mice were divided into three groups: i) 1 Day group (A); ii) 4 days group (B); and iii) 7 days group (C). Each group was comprised of four sub-groups: i) Normal control group (control); ii) atopic dermatitis (AD) group; iii) AD group treated with quinine (AD + Q); and iv) control group treated with quinine (C + Q). For treatment, 0.9% NaCl (100 μl) and quinine (100 μl;10 mg/kg) were daubed once a day, and the daubed position of the four groups was the same, taking the dermatitis lesion skin position of the AD group as a reference (Table I). At the end of the study period, animals were anesthetized with ether, and blood was collected from the retro-orbital plexus (500 μl) prior to euthanasia via cervical dislocation. Skin samples were then collected for analyses. All procedures on the mice complied with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (23). The Institutional Animal Care and Use Committee of Shenzhen University Health Science Center approved the study protocol (permit no. SCXK-2018-0002).

Histopathological analysis. Dorsal skin samples were separated and fixed in 10% neutral buffered formaldehyde for 24 h at room temperature and embedded in paraffin. Paraffin-embedded samples were cut into 4-μm thick serial sections and subjected to H&E staining for general histopathological analysis. Briefly, paraffin sections were deparaffinized with xylene at room temperature and rehydrated with a descending series (100, 95, 80 and 70%) of ethanol. Then, sections were stained with hematoxylin for 5 min, stained with 5% acetic acid for 1 min and stained with eosin for 1 min (all at room temperature). Sections were subsequently dehydrated with an ascending series (70, 80, 95 and 100%) of ethanol at room temperature. Stained sections were observed under a light microscope (magnification, x100; Olympus BX51; Olympus Corporation). The status of the epidermis in skin lesions was analyzed using Image-Pro Plus software (version 6.0; Media Cybernetics, Inc.).

Immunohistochemical detection. Tissue sections (which were prepared as described above up to the staining steps) were boiled in citrate buffer for 15 min for antigen retrieval. The slices were then incubated with 3% hydrogen peroxide for 5 min to block the endogenous peroxidase activity at room temperature. Subsequently, the sections were blocked with 5% bovine serum albumin for 30 min at 37°C. The sections were incubated overnight at 4°C with diluted antibody against FLG (1:1,000; cat. no. HPA030189; Sigma-Aldrich; Merck KGaA) and KLK7 (1:1,000; cat. no. HPA062126; Sigma-Aldrich; Merck KGaA). Following the primary antibody incubation, the tissues slides were washed with PBS and incubated with reaction enhancer solution (cat. no. PV-9001; OriGene Technologies, Inc.) at 37°C for 60 min, washed with PBS and then incubated with goat anti-rabbit IgG polymer (1:1; cat. no. PV-9001; OriGene Technologies, Inc.) at room temperature for 20 min, prior to being washed with PBS. Immunoreactivity was visualized by DAB for 1-5 min at room temperature, washed in water and re-stained with hematoxylin. Light microscopy was performed to observe the histological profiles of dorsal skin sections (magnification, x100; Eclipse E100; Nikon Corporation).

Quantification of cytokines in dorsal skin tissue and IgE in serum. Dorsal skin samples (100 mg) were homogenized in 1 ml T-PER Tissue Protein Extraction reagent. Blood samples were obtained from each treatment group after 1, 4 or 7 days of quinine treatment (24) and centrifuged at 2,000 x g for 20 min at 4°C to obtain serum. The cytokine levels of IL-4 (cat. no. ab100710; Abcam), IL-5 (cat. no. ab204523; Abcam), IL-13 (cat. no. ab219634; Abcam), TNF-α (cat. no. ab208348; Abcam) and IL-1β (cat. no. ab197742; Abcam) in skin tissue and IgE (cat. no. ab157718; Abcam) in serum were measured via ELISAs, according to the manufacturers' protocols.

Quantitative analysis of gene expression in the NF-κB signaling pathway. RT-qPCR was conducted to detect the mRNA expression levels of IκBα, IκB kinase α (IKKα) and NF-κB. The tissues samples were harvested in TRIzol® reagent for total RNA extraction. RT of the total RNA was performed with random hexamers using a PrimeScript™ RT-PCR kit according to the manufacturer's protocol. qPCR
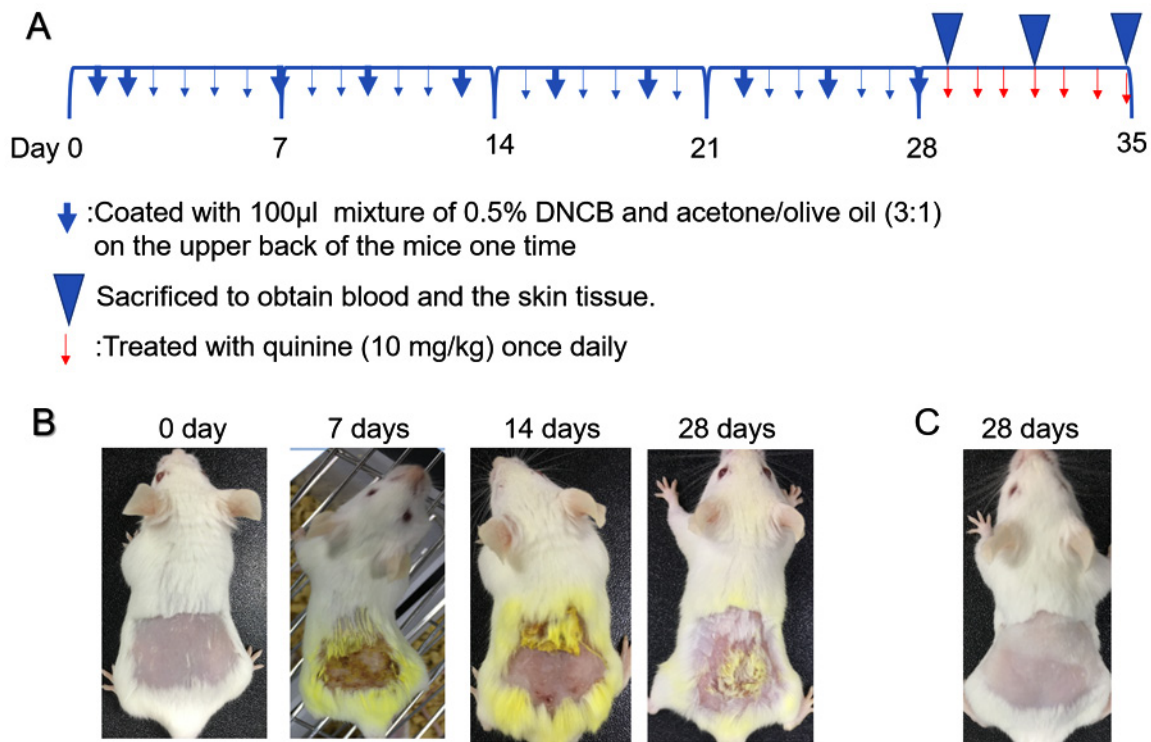
Figure 1. Experimental scheme for the induction of AD. (A) Experimental design for the induction of AD in mice and treatment with quinine. (B) Clinical features of the AD-like symptoms induced by application of a mixture of DNCB and acetone/olive oil for 0, 7, 14 and 28 days. (C) Clinical features after applying acetone/olive oil without DNCB for 28 days. AD, atopic dermatitis; DNCB, 2,4-dinitrochlorobenzene.

was then performed to determine the expression levels of the target genes using specific primers (Table II) and SYBR-Green kit according to the manufacturer's protocols. The following thermocycling conditions were used for the qPCR: 94˚C for 30 sec; followed by 40 cycles of 95˚C for 5 sec and 58˚C for 30 sec. The expression levels of IκBα, IκB kinase α (IKKα) and NF-κB were normalized to the endogenous control GAPDH using the $2^{-\Delta\Delta Cq}$ method (25).

Western blotting analysis. The back skin tissue samples (100 mg) of the mice were lysed using RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd.), homogenized in a tissue grinder and centrifuged at 12,000 x g at 4˚C for 30 min to obtain the proteins in the supernatant. Each sample was run in duplicate, and a BCA protein assay kit (cat. no. PC0020; Solarbio) was subsequently used to quantify total protein content. Equal quantities (30 μg/lane) of protein from each sample were resolved via 12% SDS-PAGE and transferred to PVDF membranes. Following blocking in 5% skimmed milk at room temperature for 1 h, membranes were incubated overnight with primary antibodies against KLK7 (1:1,000; cat. no. DF7384; Affinity Biosciences), FLG (1:500; cat. no. DF13653; Affinity Biosciences) and GAPDH (1:1,000; cat. no. 5174T; Cell Signaling Technology, Inc.). The membranes were then incubated with secondary antibodies (1:5,000; cat. no. 7074S; Cell Signaling Technology, Inc.) for 1 h at room temperature. Protein bands were visualized using an enhanced chemiluminescent western blotting kit (cat. no. P0018AS-2; Beyotime Institute of Biotechnology) according to the manufacturer's protocols. Densitometry was performed using Quantity One software (version 4.0; Bio-Rad Laboratories, Inc.).

Statistical analysis. The results are presented as the mean ± standard deviation, and the statistical comparisons of the different groups were performed using one-way ANOVA followed by Tukey's post hoc test. The Mann-Whitney test was used for comparison of two groups. α=0.05 was the statistical benchmark, and all statistical tests were two-tailed. Analyses were performed and graphs were generated using SPSS 23.0 (IBM Corp.) and GraphPad Prism 7.0 (GraphPad Software, Inc.), and all experiments were performed at least 3 times unless otherwise specified. P<0.05 was considered to indicate a statistically significant difference.

Results

Quinine improves the condition of AD-like skin lesions. To study the effect of quinine treatment on AD-like symptoms, the effects of quinine on AD-like mice were evaluated using a dermatitis severity score and the amount of scratching by the mice in a 10-min period. When treated with quinine, AD-like symptoms, including dryness, erythema, edema and excoriation, were ameliorated following quinine treatment, and quinine did not cause notable skin abnormalities in the C + Q group (Fig. 2A-D). The dermatitis severity scores and scratching frequency were significantly reduced in the AD + Q group compared with the AD-like mice (Fig. 2E and F). These results indicated that quinine relieved the clinical symptoms of AD without causing notable irritation or itching to healthy skin.

Quinine treatment reduces serum IgE levels. A major marker of AD is elevated serum total IgE levels, which are observed

Table I. Experimental design, treatment groups and analyses.

Day	Group	Sub-Group	Intervention	Analyses
1	A	Control	0.9% NaCl (100 μl)	Blood (ELISA) and skin tissue
		AD	0.9% NaCl (100 μl)	(RT-qPCR, H&E and ELISA)
		AD + Q	Quinine (100 μl; 10 mg/kg)	
		C + Q	Quinine (100 μl; 10 mg/kg)	
4	B	Control	0.9% NaCl (100 μl)	Blood (ELISA) and skin tissue
		AD	0.9% NaCl (100 μl)	(RT-qPCR, WB, IHC, H&E and
		AD + Q	Quinine (100 μl; 10 mg/kg)	ELISA)
		C + Q	Quinine (100 μl; 10 mg/kg)	
7	C	Control	0.9% NaCl (100 μl)	Blood (ELISA) and skin tissue
		AD	0.9% NaCl (100 μl)	(RT-qPCR, H&E and ELISA)
		AD + Q	Quinine (100 μl; 10 mg/kg)	
		C + Q	Quinine (100 μl; 10 mg/kg)	

AD, atopic dermatitis; RT-qPCR, reverse transcription-quantitative PCR; WB, western blotting; IHC, immunohistochemistry; C, control; Q, quinine.

Table II. Sequences of primers used in the study.

Gene	Sequence (5'→3')
IκBα	F: GGCAAGATGTAGAGGGGTATTT
	R: ATGGAAGTCATTGGTCAGGTG
IKKα	F: TCTGGAAACACTCAAGATAGACAC
	R: AAGCACAACAATGCAGGTACA
NF-κB	F: GTTATCGTTCAGTTGGTCACA
	R: ATATGCCGTCCTCACAGT
GAPDH	F: ACCCATCACCATCTTCCAGGAG
	R: GAAGGGGCGGAGATGATGAC

IKKα, IκB kinase α; F, forward; R, reverse.

in ~80% of patients with AD (8). After measuring the serum IgE levels of each mouse group, it was found that the IgE levels in the AD-like mice were increased, and that these levels decreased significantly after treatment with quinine (Fig. 2G).

Quinine reduces pathological damage to skin. As presented in Fig. 3A-C, compared with healthy mice, the epidermis in skin lesions induced by DNCB in the AD-like mice thickened, and dermal inflammatory cells had infiltrated. After quinine treatment, these pathological changes were alleviated, as DNCB-induced local thickening in the AD + Q group was significantly decreased; there were no notable changes in epidermal thickness in the C + Q group compared with the healthy controls (Fig. 3D). These indicated that quinine reduced inflammatory responses in the skin tissue of AD-like mice without inducing notable changes in healthy tissue.

Quinine treatment decreases cytokine levels in the dorsal skin. The pathophysiology of AD shows that Th1 and Th2 reactions are maladjusted, causing allergic dermatitis symptoms and leading to the release of numerous cytokines (26). NF-κB signaling pathways have reported to be involved in the production of proinflammatory cytokines in patient-derived cells, as well as in the release of tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β (27). To study whether quinine could alter the immune response, the effects of quinine on cytokine levels were evaluated in dorsal skin samples from the experimental mice. The levels of IL-4, IL-5 and IL-13 (Th2 cytokines) (28), and TNF-α and IL-1β (NF-κB-related cytokines) (27) in the skin tissue of AD-like mice were significantly elevated compared with healthy mice; after treatment with quinine, the levels of IL-4, IL-5, IL-13, TNF-α and IL-1β were significantly decreased, whereas the immune response in the skin of the C + Q group was unchanged (Fig. 3E-I).

Quinine treatment improves skin barrier function. FLG serves the protective function of maintaining the skin barrier, and KLK7 is a serine protease involved in the proteolysis of extracellular corneal linker components, which results in desquamation (29). Increased KLK7 levels have been shown to induce spontaneous itching in mice (30). To further investigate the protective function of quinine on the skin barrier, western blotting and immunohistochemistry were performed. Reduced FLG expression and increased KLK7 expression in AD-like mice suggested that the skin barrier of the AD-like mice was damaged. Following quinine treatment, FLG expression was increased and KLK7 expression was decreased in the AD + Q group compared with the AD group, indicating that the skin barrier was restored (Fig. 4A-C). The expression of FLG and KLK7 was also investigated in skin lesions via immunohistochemistry, and the results were consistent with the western blot analysis (Fig. 4D and E), suggesting that quinine exhibited a protective effect on the skin barrier.

Quinine inhibits the expression of genes involved in the NF-κB signaling pathway. To study the mechanism via which quinine
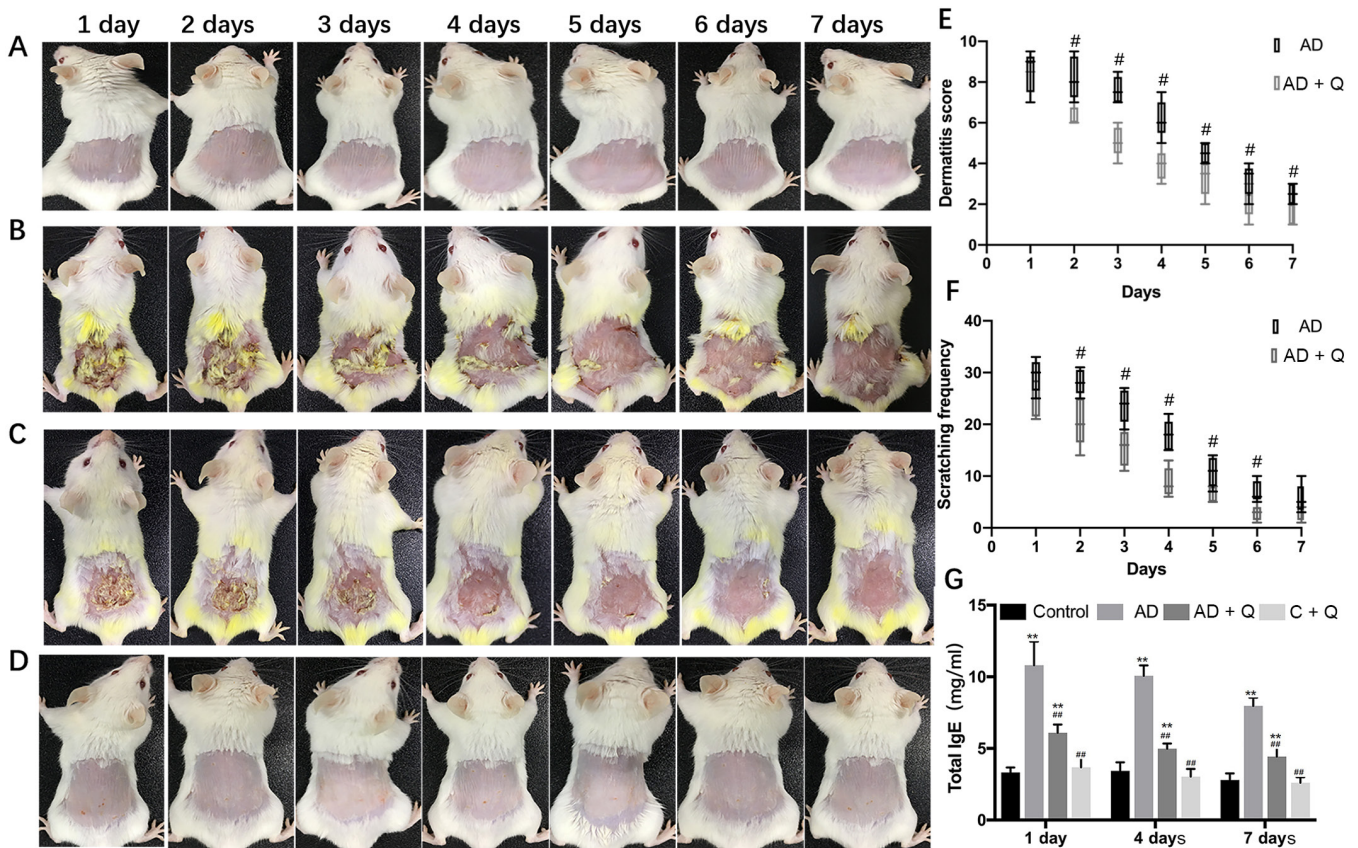
Figure 2. Therapeutic effects of quinine on AD-like symptoms in mice. (A) Skin condition in the healthy control group. (B) Clinical features of the AD-like mouse group from 1 to 7 days. (C) AD-like mouse treated with quinine for 7 days. (D) Healthy mice treated with quinine for 7 days. (E) Dermatitis severity scores for the skin lesions. (F) Scratching frequency. (G) Total IgE levels in serum. Data are presented as the mean ± standard deviation. **P<0.001 vs. control; $^{#}$P<0.05, $^{##}$P<0.001 vs. AD. AD, atopic dermatitis; AD + Q, AD group treated with quinine; C + Q, control group treated with quinine; IgE, immunoglobulin E.

ameliorates inflammation in AD-like mice, the expression levels of genes associated with the NF-κB signaling pathway were evaluated, including IκBα, IKKα and NF-κB. It has been reported that IκBα is a negative regulator of NF-κB (31); however, the present results found that the levels of IκBα, IKKα and NF-κB mRNA expression were significantly increased in AD-like mice (Fig. 4F-H). After quinine treatment, the expression of IκBα, IKKα and NF-κB mRNA was decreased significantly compared with the AD group (Fig. 4F-H), which may be involved in inhibiting the development of inflammation. These results suggested that quinine reduced activation of the NF-κB signaling pathway at the mRNA level, thus serving a potential role in the treatment of AD.

Discussion

Quinine, an alkaloid derived from the bark of cinchona in the Andean forests, was the first effective treatment for malaria (32). Studies have reported that the TAS2R agonist can inhibit IgE secretion in a mouse model of asthma, thus relieving the clinical symptoms (12,13). Therefore, the present study aimed to evaluate the therapeutic effects of quinine on AD-like mice. The results demonstrated that quinine treatment effectively attenuated AD-like symptoms in mice, reducing the dermatitis severity score, serum IgE levels and the infiltration of inflammatory cells, as well as alleviating the pathological damage, inhibiting the expression of genes related

to the NF-κB signaling pathway, reducing the inflammatory response and promoting the expression of the skin-protective protein FLG. It was also found that quinine reduced cytokine levels in the AD-like mice. These results suggested that the topical application of quinine may provide a novel therapeutic regimen for the treatment AD, as well as indicating that quinine does not cause notable harm to healthy skin.

In the present study, it was revealed that quinine increased the levels of Th2-related factors and a number of cytokines associated with NF-κB signaling in the dorsal skin lesions of AD-like mice. When treated with quinine, the cytokine levels decreased. Previous studies had shown that the pathophysiology of the skin changes in AD, including dysregulation of Th1 and Th2 responses, characterized by IL-4, IL-5 and IL-13-mediated allergic dermatitis (26,28). Elevated levels of Th2-related cytokines have been detected in the skin lesions of patients with AD, and these elevated levels have been associated with elevated circulating IgE levels (33).

AD primarily consists of skin damage. The main reason for the onset of the disease is skin barrier defects; one of the important proteins involved is the skin structural protein FLG (34,35). Naeem *et al* (36) demonstrated that increasing FLG expression could alleviate AD-like skin lesions. The present study demonstrated that FLG expression was reduced in the skin lesions of AD-like mice and that quinine treatment increased FLG levels, which may be an important factor underlying the improvement in the skin lesions. Studies have
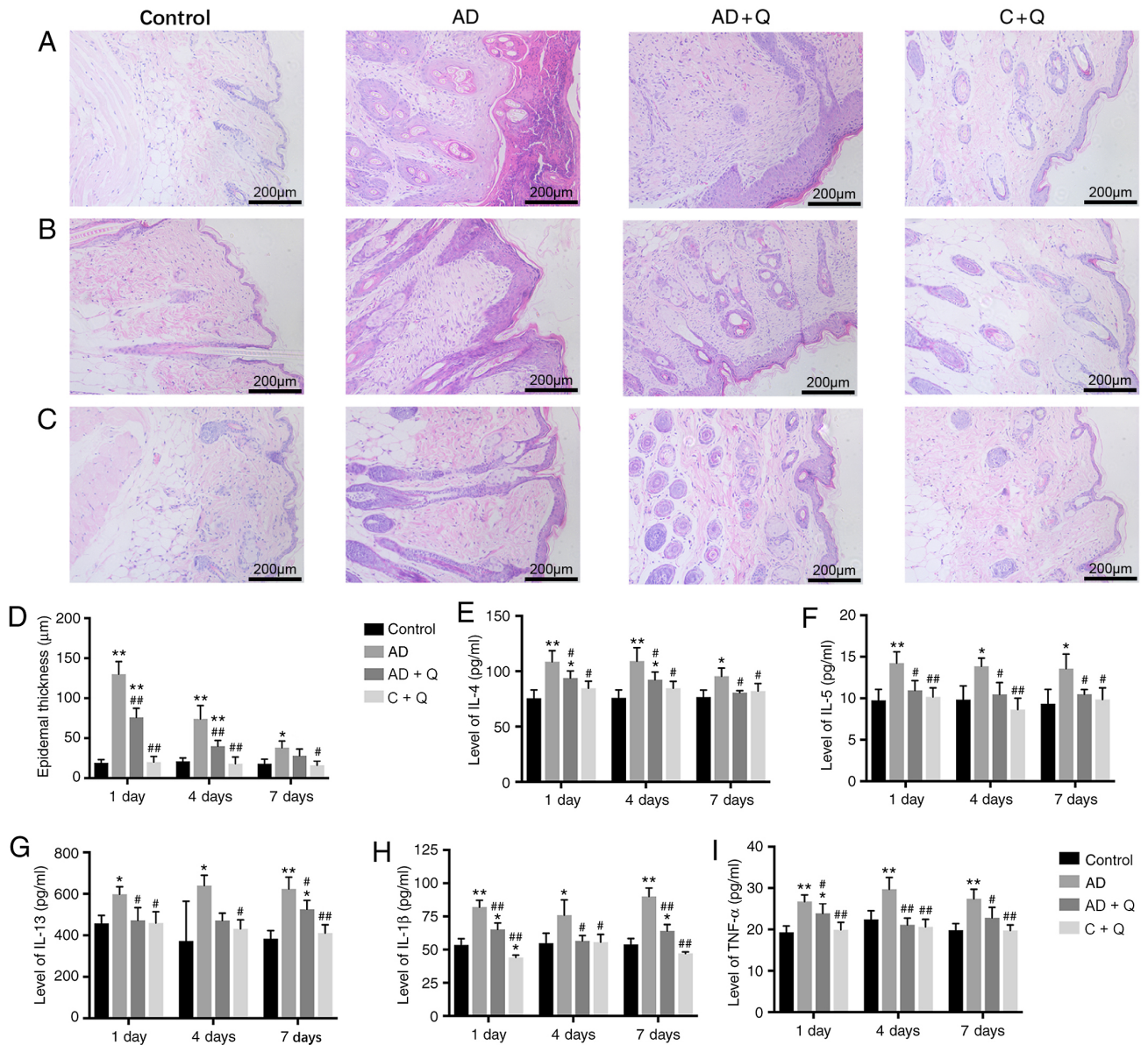
Figure 3. Histopathological analysis of the back skin tissues. Dorsal skin sections were stained with H&E (magnification, x100). (A) Skin tissues following treatment for 1 day. (B) Skin tissues following treatment for 4 days. (C) Skin tissues following treatment for 7 days. (D) Epidermal thickness in the dorsal skin. Levels of (E) IL-4, (F) IL-5, (G) IL-13, (H) IL-1β and (I) TNF-α in dorsal skin tissues. Data are presented as the mean ± standard deviation. *P<0.05, **P<0.001 vs. control; $^#$P<0.05, $^{##}$P<0.001 vs. AD. AD, atopic dermatitis; AD + Q, AD group treated with quinine; C + Q, control group treated with quinine; IL, interleukin; TNF, tumor necrosis factor.

shown that the skin lesions of patients with AD contain high KLK levels, which may be related to the itching associated with AD (37). Experiments with mice have shown that KLK overexpression in the skin can spontaneously induce AD-like disease (29,38). In the present study, it was found that the scratching frequency of the AD-like mice was significantly reduced after treatment with quinine compared with the untreated AD-like mice. To further study the mechanism, KLK7 expression in the dorsal skin was examined. The results showed that KLK7 expression decreased in the skin lesions of AD-like mice following quinine treatment.

To further elucidate the mechanisms underlying the anti-AD effects of quinine, NF-κB mRNA expression was measured in the dorsal skin of each group. NF-κB expression was increased in the AD-like mice; when treated with

quinine, this increase was attenuated. NF-κB is an important transcription factor that serves central roles in the occurrence and development of acute and chronic inflammatory diseases (39). NF-κB signaling pathways have been reported to be involved in the production of proinflammatory cytokines in patient-derived cells (40). To verify the effect and mechanism of quinine in AD, we the mRNA expression levels of IκBα, IKKα and NF-κB, which are all involved in NF-κB signaling pathway, were investigated. Upon receiving an extracellular signal, which results in the phosphorylation, ubiquitination, and degradation of IκBα, NF-κB is permitted to enter the nucleus (31,41). Newly synthesized IκBα enters the nucleus and strips NF-κB from DNA, thereby terminating the signal response through an unknown mechanism, a process in which a transient ternary complex between NF-κB, its cognate DNA
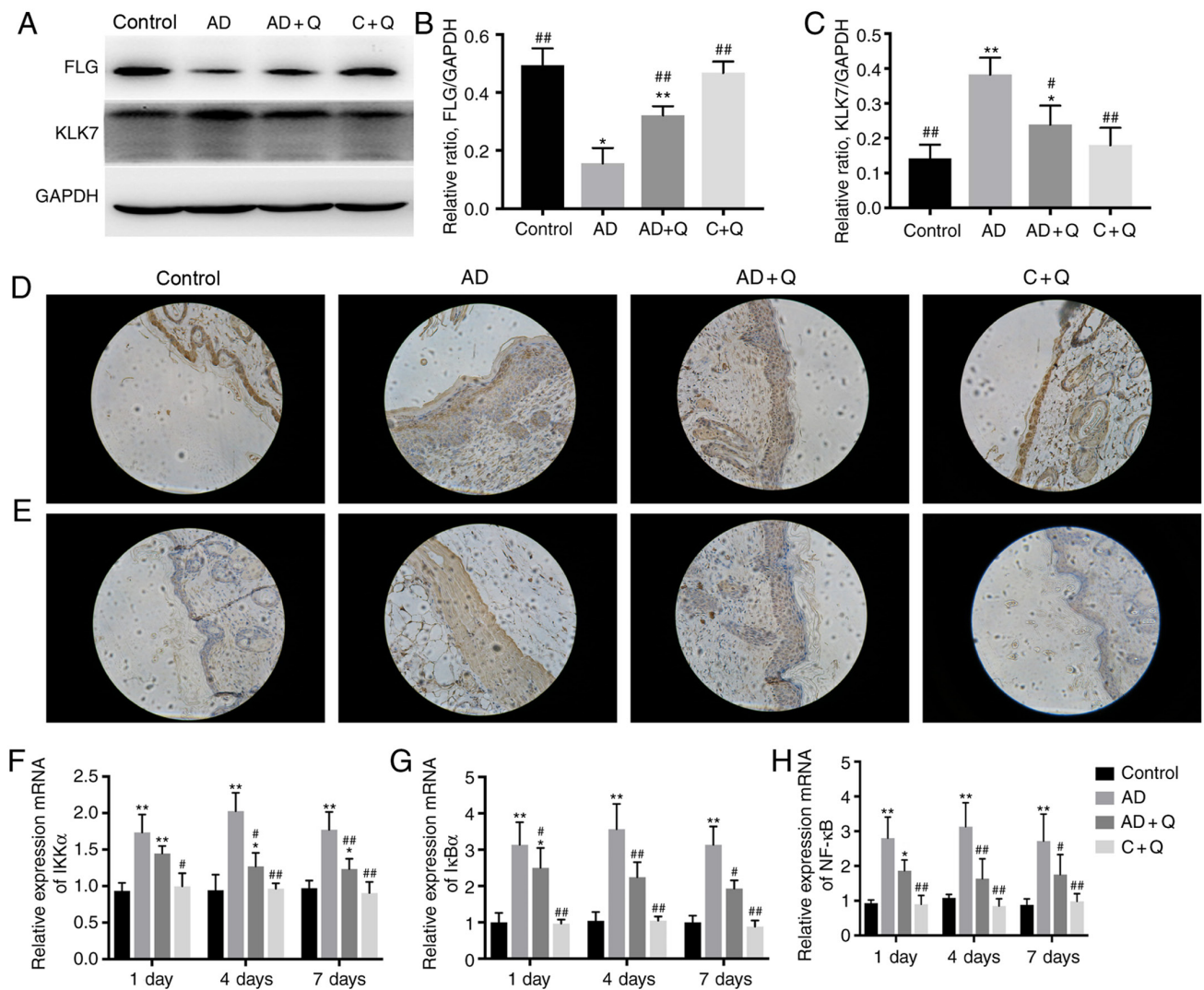
Figure 4. Effects of quinine on protein and mRNA expression in AD-like mice. (A) Protein expression of FLG and KLK7 evaluated via western blotting. (B) Relative ratio of FLG/GAPDH. (C) Relative ratio of KLK7/GAPDH. (D) Protein expression of FLG evaluated via immunohistochemistry. (E) Protein expression of KLK7 evaluated via immunohistochemistry. mRNA expression of (F) IκBα, (G) IKKα and (H) NF-κB determined via reverse transcription-quantitative PCR analysis. Data are presented as the mean ± standard deviation. *P<0.05, **P<0.001 vs. control; #P<0.05, ##P<0.001 vs. AD. AD, atopic dermatitis; AD + Q, AD group treated with quinine; C + Q, control group treated with quinine; FLG, filaggrin; KLK7, kallikrein-7; IKKα, IκB kinase α.

κB sequence and IκBα is observed (31,41,42). Interestingly, the present results showed that the expression of IκBα and NF-κB increased simultaneously in the skin lesion of AD-like mice, and concurrently decreased following administration of quinine. The present data were inconsistent with previous reports which indicated that IκBα was a negative regulator of NF-κB (31,41,42). However, the present study only investigated mRNA expression levels; thus, further research will aim to clarify the regulatory role of IκBα on the expression levels of proteins associated with the NF-κB signaling pathway in AD.

Taken together, the present results indicated that quinine suppressed Th2-related cytokines (IL-4, IL-5 and IL-13), IL-1β and TNF-α by inhibiting the activity of NF-κB signaling pathways in a DNCB-induced mouse model of AD. Quinine may therefore be an effective treatment for AD. In future studies, inhibitors of proteins in the NF-κB signaling pathway will be used to further determine the mechanisms underlying the effects of quinine in the treatment of AD.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LW and SC conceived and designed this study. QZ, HJJ, ML, XCL and MRZ performed the *in vivo* studies and collected the data. YSL and JKH analyzed the data and interpreted the results of the experiments. LW and SC confirm the authenticity of all the raw data. XCL and MRZ prepared the figures. SC and QZ revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Institutional Animal Care and Use Committee of Shenzhen University Health Science Center approved the study protocol (permit no. SCXK-2018-0002).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Nutten S: Atopic dermatitis: Global epidemiology and risk factors. Ann Nutr Metab 66 (Suppl 1): S8-S16, 2015.
2. Eichenfield LF, Tom WL, Chamlin SL, Feldman SR, Hanifin JM, Simpson EL, Berger TG, Bergman JN, Cohen DE, Cooper KD, *et al*: Guidelines of care for the management of atopic dermatitis: Section 1. Diagnosis and assessment of atopic dermatitis. J Am Acad Dermatol 70: 338-351, 2014.
3. Weidinger S and Novak N: Atopic dermatitis. Lancet 387: 1109-1122, 2016.
4. De Benedetto A, Kubo A and Beck LA: Skin barrier disruption: A requirement for allergen sensitization? J Invest Dermatol 132: 949-963, 2012.
5. Leung DY: New insights into atopic dermatitis: Role of skin barrier and immune dysregulation. Allergol Int 62: 151-161, 2013.
6. Leung DY: Atopic dermatitis: New insights and opportunities for therapeutic intervention. J Allergy Clin Immunol 105: 860-876, 2000.
7. Han H, Roan F and Ziegler SF: The atopic march: Current insights into skin barrier dysfunction and epithelial cell-derived cytokines. Immunol Rev 278: 116-130, 2017.
8. Kasperkiewicz M, Schmidt E, Ludwig RJ and Zillikens D: Targeting IgE antibodies by immunoadsorption in atopic dermatitis. Front Immunol 9: 254, 2018.
9. Jeruzal-Świątecka J, Fendler W and Pietruszewska W: Clinical role of extraoral bitter taste receptors. Int J Mol Sci 21: 5156, 2020.
10. Workman AD, Palmer JN, Adappa ND and Cohen NA: The role of bitter and sweet taste receptors in upper airway immunity. Curr Allergy Asthma Rep 15: 72, 2015.
11. Deshpande DA, Wang WCH, McIlmoyle EL, Robinett KS, Schillinger RM, An SS, Sham JS AND Liggett SB: Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. Nat Med 16: 1299-1304, 2010.
12. Orsmark-Pietras C, James A, Konradsen JR, Nordlund B, Söderhäll C, Pulkkinen V, Pedroletti C, Daham K, Kupczyk M, Dahlén B, *et al*: Transcriptome analysis reveals upregulation of bitter taste receptors in severe asthmatics. Eur Respir J 42: 65-78, 2013.
13. Ekoff M, Choi JH, James A, Dahlén B, Nilsson G and Dahlén SE: Bitter taste receptor (TAS2R) agonists inhibit IgE-dependent mast cell activation. J Allergy Clin Immunol 134: 475-478, 2014.
14. Tomic S: L'Analyse chimique des végétaux: Le cas du quinquina. Ann Sci 58: 287-309, 2001.
15. Achan J, Talisuna AO, Erhart A, Yeka A, Tibenderana JK, Baliraine FN, Rosenthal PJ and D'Alessandro U: Quinine, an old anti-malarial drug in a modern world: Role in the treatment of malaria. Malar J 10: 144, 2011.
16. Tse EG, Korsik M and Todd MH: The past, present and future of anti-malarial medicines. Malar J 18: 93, 2019.
17. Gachelin G, Garner P, Ferroni E, Tröhler U and Chalmers I: Evaluating Cinchona bark and quinine for treating and preventing malaria. J R Soc Med 110: 31-40, 2017.
18. Sharma P, Yi R, Nayak AP, Wang N, Tang F, Knight MJ, Pan S, Oliver B and Deshpande DA: Bitter taste receptor agonists mitigate features of allergic asthma in mice. Sci Rep 7: 46166, 2017.
19. Shaw L, Mansfield C, Colquitt L, Lin C, Ferreira J, Emmetsberger J and Reed DR: Personalized expression of bitter 'taste' receptors in human skin. PLoS One 13: e0205322, 2018.
20. Yang H, Jung EM, Ahn C, Lee GS, Lee SY, Kim SH, Choi IG, Park MJ, Lee SS, Choi DH and Jeung EB: Elemol from chamaecyparis obtusa ameliorates 2,4-dinitrochlorobenzene-induced atopic dermatitis. Int J Mol Med 36: 463-472, 2015.
21. Caglayan Sozmen S, Karaman M, Cilaker Micili S, Isik S, Arikan Ayyildiz Z, Bagriyanik A, Uzuner N and Karaman O: Resveratrol ameliorates 2,4-dinitrofluorobenzene-induced atopic dermatitis-like lesions through effects on the epithelium. PeerJ 4: e1889, 2016.
22. Yamamoto M, Haruna T, Yasui K, Takahashi H, Iduhara M, Takaki S, Deguchi M and Arimura A: A novel atopic dermatitis model induced by topical application with dermatophagoides farinae extract in NC/Nga mice. Allergol Int 56: 139-148, 2007.
23. National Institutes of Health. Guide for the care and use of laboratory animals. 7th edition. Washington DC, National Academy Press, 1996.
24. Jin W, Huang W, Chen L, Jin M, Wang Q, Gao Z and Jin Z: Topical application of JAK1/JAK2 inhibitor momelotinib exhibits significant anti-inflammatory responses in DNCB-induced atopic dermatitis model mice. Int J Mol Sci 19: 3973, 2018.
25. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
26. Grewe M, Bruijnzeel-Koomen CA, Schöpf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T and Krutmann J: A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. Immunol Today 19: 359-361, 1998.
27. Yatoo MI, Gopalakrishnan A, Saxena A, Parray OR, Tufani NA, Chakraborty S, Tiwari R, Dhama K and Iqbal HMN: Anti-inflammatory drugs and herbs with special emphasis on herbal medicines for countering inflammatory diseases and disorders-a review. Recent Pat Inflamm Allergy Drug Discov 12: 39-58, 2018.
28. Lambrecht BN, Hammad H and Fahy JV: The cytokines of asthma. Immunity 50: 975-991, 2019.
29. Igawa S, Kishibe M, Minami-Hori M, Honma M, Tsujimura H, Ishikawa J, Fujimura T, Murakami M and Ishida-Yamamoto A: Incomplete KLK7 secretion and upregulated LEKTI expression underlie hyperkeratotic stratum corneum in atopic dermatitis. J Invest Dermatol 137: 449-456, 2017.
30. Guo CJ, Mack MR, Oetjen LK, Trier AM, Council ML, Pavel AB, Guttman-Yassky E, Kim BS and Liu Q: Kallikrein 7 promotes atopic dermatitis-associated itch independently of skin inflammation. J Invest Dermatol 140: 1244-1252.e4, 2020.
31. Mukherjee SP, Quintas PO, McNulty R, Komives EA and Dyson HJ: Structural characterization of the ternary complex that mediates termination of NF-κB signaling by IκBα. Proc Natl Acad Sci USA 113: 6212-6217, 2016.
32. Shanks GD: Historical review: Problematic malaria prophylaxis with quinine. Am J Trop Med Hyg 95: 269-272, 2016.
33. Jeong CW, Ahn KS, Rho NK, Park YD, Lee DY, Lee JH, Lee ES and Yang JM: Differential in vivo cytokine mRNA expression in lesional skin of intrinsic vs extrinsic atopic dermatitis patients using semiquantitative RT-PCR. Clin Exp Allergy 33: 1717-1724, 2003.
34. Callard RE and Harper JI: The skin barrier, atopic dermatitis and allergy: A role for Langerhans cells? Trends Immunol 28: 294-298, 2007.
35. Jakasa I, de Jongh CM, Verberk MM, Bos JD and Kezić S: Percutaneous penetration of sodium lauryl sulphate is increased in uninvolved skin of patients with atopic dermatitis compared with control subjects. Br J Dermatol 155: 104-109, 2006.

36. Naeem AS, Tommasi C, Cole C, Brown SJ, Zhu Y, Way B, Willis Owen SA, Moffatt M, Cookson WO, Harper JI, *et al*: A mechanistic target of rapamycin complex 1/2 (mTORC1)/V-Akt murine thymoma viral oncogene homolog 1 (AKT1)/cathepsin H axis controls filaggrin expression and processing in skin, a novel mechanism for skin barrier disruption in patients with atopic dermatitis. J Allergy Clin Immunol 139: 1228-1241, 2017.
37. Komatsu N, Saijoh K, Kuk C, Liu AC, Khan S, Shirasaki F, Takehara K and Diamandis EP: Human tissue kallikrein expression in the stratum corneum and serum of atopic dermatitis patients. Exp Dermatol 16: 513-519, 2007.
38. Briot A, Deraison C, Lacroix M, Bonnart C, Robin A, Besson C, Dubus P and Hovnanian A: Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. J Exp Med 206: 1135-1147, 2009.
39. Barnes PJ and Karin M: Nuclear factor-kappaB: A pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 336: 1066-1071, 1997.
40. Fisher CL, Pineault N, Brookes C, Helgason CD, Ohta H, Bodner C, Hess JL, Humphries RK and Brock HW: Loss-of-function additional sex combs like 1 mutations disrupt hematopoiesis but do not cause severe myelodysplasia or leukemia. Blood 115: 38-46, 2010.
41. Sue SC, Alverdi V, Komives EA and Dyson HJ: Detection of a ternary complex of NF-kappaB and IkappaBalpha with DNA provides insights into how IkappaBalpha removes NF-kappaB from transcription sites. Proc Natl Acad Sci USA 108: 1367-1372, 2011.
42. Hayden MS and Ghosh S: Signaling to NF-kappaB. Genes Dev 18: 2195-2224, 2004.