



Contents lists available at SciVerse ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm



Inhibitory effects of *Chelidonium majus* extract on atopic dermatitis-like skin lesions in NC/Nga mice

Gabsik Yang, Kyungjin Lee, Mi-Hwa Lee, So-Hyung Kim, In-Hye Ham, Ho-Young Choi*

Department of Herbology, College of Oriental Medicine, Kyung-Hee University, Seoul 130-701, Republic of Korea

ARTICLE INFO

Article history:

Received 24 March 2011
Received in revised form 12 August 2011
Accepted 16 September 2011
Available online xxx

Keywords:

Chelidonium majus
Atopic dermatitis
NC/Nga
Immunoglobulin E
DNCEB

ABSTRACT

Aim of the study: *Chelidonium majus* (CM) has traditionally been used for treatment of various inflammatory diseases including atopic dermatitis (AD). However its action on atopic dermatitis (AD) is unclear. Therefore, we investigated the effect of CM on AD using NC/Nga mice as an AD model.

Materials and methods: The effect of CM on 1-chloro-2,4-dinitrobenzene (DNCEB) induced NC/Nga mice was evaluated by examining skin symptom severity, itching behavior, ear thickness, levels of serum immunoglobulin E (IgE), tumor necrosis factor- α (TNF- α), and interleukin-4 (IL-4), skin histology.

Results: The CM significantly reduced the total clinical severity score, itching behavior, ear thickness and the level of serum IgE in AD mouse model. CM not only decreased TNF- α but also IL-4.

Conclusion: These results suggest that CM may be a potential therapeutic modality for AD.

© 2011 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Atopic dermatitis (AD) is a common disease affecting both children and adults. AD develops from a complex interplay between environmental, genetic, immunologic and biochemical factors (Pugliarello et al., 2010). Chronic inflammation in AD is associated with elevated levels of serum immunoglobulin E (IgE) and eosinophilia in the tissues and peripheral blood, and frequently occurs in response to environmental allergens such as house-dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Leung, 2000; Matsuoka et al., 2003).

The NC/Nga mouse is the most extensively studied animal model of AD. The NC/Nga strain originated from Japanese fancy mice and was established as an inbred strain by Kondo et al. in 1957. These mice spontaneously develop AD-like eczematous skin lesions when kept under conventional conditions, but not when maintained under specific pathogen-free (SPF) conditions (Shiohara et al., 2004). Clinical symptoms begin with itching, erythema, hemorrhage, scaling, dryness, and alopecia at the age of 8 weeks. These

eczematous skin lesions are typically observed on the face, nose, ears, neck and back, suggesting that they are caused by hind limb scratching. The clinical severity of the dermatitis, as determined by a scoring system which has been established for human AD, increases with age and reaches a maximum at around 17 weeks of age (Matsuda et al., 1997).

Chelidonium majus L. is a plant which grows in the wild in Southern and Central Europe, part of Asia, North America and in the Azores archipelago (Tin-Wa et al., 1972; Colombo and Bosisio, 1996). *Chelidonium majus* L. (family Papaveraceae), or greater celandine, is an important plant in western phytotherapy and in traditional Chinese medicine. *Chelidonium majus* L. has multiple applications in folk medicine because of its anti-tumoral, cytotoxic, anti-inflammatory and anti-microbial activities (Kim et al., 1969; Saglam and Arar, 2003). Crude extracts of *Chelidonium majus* as well as purified compounds derived from it exhibit a broad spectrum of biological activities (anti-inflammatory, antimicrobial, antitumoral, analgesic, hepatoprotective) that support some of the traditional uses of *Chelidonium majus* (Gilca et al., 2010). The plant contains, as major secondary metabolites, isoquinoline alkaloids, such as sanguinarine, chelidonine, chelerythrine, berberine and coptisine. Isoquinoline alkaloids have anti-inflammatory activity (Kupeli et al., 2002). Sanguinarine, chelerythrine and quaternary benzophenanthridine fraction were screened for their anti-inflammatory activity in assays involving carrageenan-induced rat paw edema. Sanguinarine showed a higher anti-inflammatory activity than chelerythrine, which could be explained with the different oxygen electrodonating substituents (Lenfeld et al., 1981). In spite of the traditional usage

Abbreviations: AD, atopic dermatitis; CM, *Chelidonium majus*; IL-4, interleukin-4; IgE, immunoglobulin E; TNF- α , tumor necrosis factor- α ; DNCEB, 1-chloro-2,4-dinitrobenzene; NOR, normal group; CON, control group; CMP 1, *Chelidonium majus* Methanol Extract 100 mg/kg, p.o. group; CMP 4, *Chelidonium majus* Methanol Extract 400 mg/kg, p.o. group; CMS 1, *Chelidonium majus* Methanol Extract 1% (200 μ l) smear group; CMS 2, *Chelidonium majus* Methanol Extract 2% (200 μ l) smear group.

* Corresponding author. Tel.: +82 2 961 9372; fax: +82 2 965 9372.

E-mail addresses: hychoi@khu.ac.kr, dostudy@naver.com, gabsigi@hanmail.net (H.-Y. Choi).

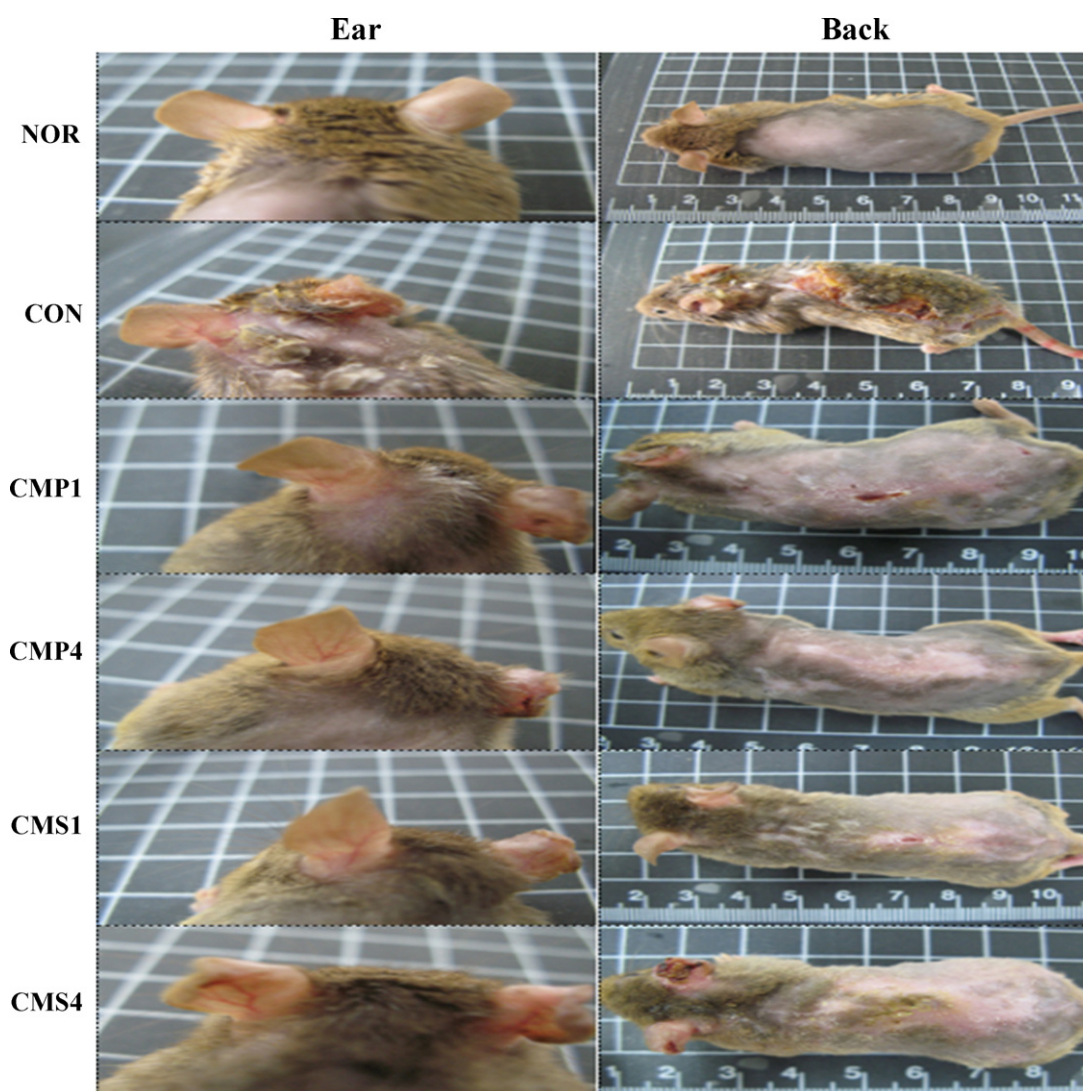


Fig. 1. Clinical features of NC/Nga mice. The photograph was taken on the 6 weeks after sensitization.

of *Chelidonium majus* to treat allergic disease, its mechanism has not been fully verified using scientific tools. Under the circumstances, we investigated the anti-allergic effects of *Chelidonium majus* extract on NC/Nga mice as a model of DNCB-induced atopic dermatitis.

2. Materials and methods

2.1. Preparation of CM extract

Chelidonium majus as a dried herb was collected from Gunwi, Kyungpook Province, South Korea and authenticated by H.-Y. Choi, College of Oriental Medicine, Kyung Hee University. A voucher specimen was deposited at the Herbarium of the College of Oriental Medicine, Kyung Hee University. The dried aerial part of *Chelidonium majus* (5 kg) was extracted twice with 70% ethanol (with 2 h reflux), and extract was then concentrated under reduced pressure. The decoction was filtered, lyophilized, and serially stored at 4 °C. The yield of dried extract from starting crude materials was about 6.64% (w/w). The extract was dissolved in saline or phosphate-buffered saline (PBS) and then filtered through a 0.22- μ m syringe filter for experiments.

2.2. Experimental animals

Female 5-week-old NC/Nga mice were purchased from SLC, Inc. (Hamamatsu, Japan) and were maintained for 1 week prior to experiments. They were housed in an air-conditioned animal room with a 12-h light/12-h dark cycle at a temperature of 22 ± 1 °C and humidity of $50 \pm 10\%$. Mice were provided with a laboratory diet and water *ad libitum*. All experimental protocols for animal care involving the use of animals were conducted in accordance with National Institutes of Health (NIH) Guidelines and approved by the Committee on Animal Care of our institute.

2.3. Induction of dermatitis

1-Chloro-2,4-dinitrobenzene (DNCB; Sigma-Aldrich, St Louis, MO, USA) was used to induce dermatitis in all three groups of NC/Nga mice through repeated cutaneous application as previously described. Allergic dermatitis on NC/Nga mice was induced according to the protocol reported by Gao et al. (2005) with a slight modification. Briefly, DNCB in an acetone/olive oil mixture (1:3) was used. Dorsal skin regions were shaved with an electric razor and these and the glabrous ears were sensitized epicutaneously with 200 μ l of a 1% DNCB solution. Controlled dermatitis

Table 1Time-dependent clinical score of the atopic dermatitis-like NC/Nga mouse after the administration with *Chelidonium majus*.

Clinical score	Dose	0 day	7th day	14th day	28th day	42nd day
NOR		0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.5	0.8 ± 1.3	1.0 ± 1.6
CON		0.0 ± 0.0	6.2 ± 0.8 ^{##}	11.3 ± 1.5 ^{##}	7.3 ± 0.8 ^{##}	7.3 ± 0.8 ^{##}
CMP 1	p.o.	100 mg/kg	0.0 ± 0.0 ^{**}	5.3 ± 0.8	10.5 ± 2.1 ^{**}	5.0 ± 1.3
CMP 4	p.o.	400 mg/kg	0.0 ± 0.0 ^{**}	4.8 ± 1.5 ^{**}	9.8 ± 2.9 [*]	5.8 ± 3.2
CMS 1	Smear	1%	0.0 ± 0.0 ^{**}	6.5 ± 0.6 ^{**}	12.3 ± 0.8 ^{**}	6.6 ± 0.8
CMS 2	Smear	2%	0.0 ± 0.0 ^{**}	5.2 ± 1.0 ^{**}	10.3 ± 2.0 ^{**}	6.5 ± 0.6

Data are presented with mean ± standard deviations (N=5). Data were analyzed using analysis of variance with Student's *t*-test. NOR: normal group; CON: control group; CMP 1: 100 mg/kg, p.o. group; CMP 4: 400 mg/kg, p.o. group; CMS 1: 1% (200 µl) smear group; CMS 2: 2% (200 µl) smear group.

* *P* < 0.05 versus control.

** *P* < 0.01 versus control.

P < 0.01 control versus normal.

was induced by topically applying 0.1% DNCB dissolved in acetone/olive oil (1:3), to the hair-removed back of mice 3 times per week (Monday, Wednesday and Friday) for 6 weeks. Mice were then housed for 3 days without any further treatment. In the case of the CM-treated group, CMP was orally administered at a dose of 100 or 400 mg/kg (6 times per week for 6 weeks) in their drinking water and CMS was topically applied at a dose of 1% or 2%. Control animals received the vehicle. Ear thickness was measured using digital thickness gauge immediately before CM treatment. At day 42, final ear thickness was measured. Mice were sacrificed on the day of the experiment (on 42 days after first applying the DNCB) and blood was collected from the *vena cava*. Skin tissues from the backs of mice were excised and subjected to histological examination.

2.4. Evaluation of skin severity

The severity of dermatitis was evaluated twice a week, just before the application of DNCB and/or CM. The development of (1) erythema/hemorrhage, (2) dryness/scaling, (3) edema, (4) erosion/excoriation was scored as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). The sum of the individual scores was taken as the dermatitis score (Matsuda et al., 1997). The intensity of score was evaluated as mild (<20%), moderate (20–60%), and severe (>60% of skin area).

Table 2Scratching behavior profiles of the atopic dermatitis-like NC/Nga mouse after the administration with *Chelidonium majus*.

	Dose	Administration route	No. of scratching behavior (times)
NOR	–	–	32.2 ± 1.5
CON	–	–	63.0 ± 2.9 ^{##}
CMP 1	100 mg/kg	p.o.	39.2 ± 6.5 ^{**}
CMP 4	400 mg/kg	p.o.	39.0 ± 2.2 ^{**}
CMS 1	1%	Smear	47.8 ± 4.4 ^{**}
CMS 2	2%	Smear	47.0 ± 4.2

Data are presented with mean ± standard deviations (N=5). Data were analyzed using analysis of variance with Student's *t*-test. NOR: normal group; CON: control group; CMP 1: 100 mg/kg, p.o. group; CMP 4: 400 mg/kg, p.o. group; CMS 1: 1% (200 µl) smear group; CMS 2: 2% (200 µl) smear group.

** *P* < 0.01 versus control.

P < 0.01 control versus normal.

2.5. Evaluation of itching behavior

Mice were placed individually in a clear plastic cage and their behavior was watched for 30 min 9 weeks after sensitization. Scratching of the rostral back and biting of the caudal back were observed; scratching movements by the hind paw was defined as a scratching bout which ended when the mice either licked its hind paw or placed its hind paw back on the floor and a series of one or

Table 3Time-dependent ear thickness change of the atopic dermatitis-like NC/Nga mouse after the administration with *Chelidonium majus*.

Ear thickness	Dose	0 day (%)	7th day (%)	14th day (%)	28th day (%)	42nd day (%)
NOR		19.0 ± 1.9	19.9 ± 1.9	20.8 ± 1.6	21.4 ± 2.3	22.1 ± 0.9
CON		19.0 ± 1.4	27.2 ± 2.2 ^{##}	69.4 ± 1.1 ^{##}	83.9 ± 5.3 ^{##}	100.0 ± 5.1 ^{##}
CMP 1	p.o.	100 mg/kg	18.5 ± 1.0 ^{**}	24.2 ± 1.4 ^{**}	50.2 ± 3.3 ^{**}	58.1 ± 3.5 ^{**}
CMP 4	p.o.	400 mg/kg	19.4 ± 2.3 ^{**}	24.1 ± 1.7 ^{**}	48.4 ± 2.2 ^{**}	55.4 ± 3.6 ^{**}
CMS 1	Smear	1%	17.1 ± 1.3 ^{**}	24.2 ± 2.5 ^{**}	64.1 ± 6.3 ^{**}	72.9 ± 4.9 ^{**}
CMS 2	Smear	2%	17.8 ± 1.3 ^{**}	23.2 ± 1.6 ^{**}	63.8 ± 6.1 ^{**}	73.3 ± 5.5 ^{**}

Data are presented with mean ± standard deviations (N=5). Data were analyzed using analysis of variance with Student's *t*-test. NOR: normal group; CON: control group; CMP 1: 100 mg/kg, p.o. group; CMP 4: 400 mg/kg, p.o. group; CMS 1: 1% (200 µl) smear group; CMS 2: 2% (200 µl) smear group.

** *P* < 0.01 versus control.

P < 0.01 control versus normal.

Table 4Serum IgE, TNF-α and IL-4 profiles of the atopic dermatitis-like NC/Nga mouse after the administration with *Chelidonium majus*.

	Dose	Administration route	IgE (ng/mL)	TNF-α (pg/mL)	IL-4 (pg/mL)
NOR	–	–	77.4 ± 6.6	24.6 ± 6.6	3.4 ± 0.5
CON	–	–	106.8 ± 13.4 ^{##}	61.6 ± 9.3 ^{##}	46.7 ± 21.5 ^{##}
CMP 1	100 mg/kg	p.o.	84.2 ± 3.2 ^{**}	–	4.3 ± 0.7 [*]
CMP 4	400 mg/kg	p.o.	77.6 ± 8.8 ^{**}	–	4.9 ± 1.6 [*]
CMS1	1%	Smear	–	49.2 ± 7.7	4.6 ± 1.0 [*]
CMS2	2%	Smear	–	48.5 ± 6.1 [*]	4.7 ± 0.6 [*]

Data are presented with mean ± standard deviations (N=5). Data were analyzed using analysis of variance with Student's *t*-test. NOR: normal group; CON: control group; CMP 1: 100 mg/kg, p.o. group; CMP 4: 400 mg/kg, p.o. group; CMS 1: 1% (200 µl) smear group; CMS 2: 2% (200 µl) smear group.

* *P* < 0.05 versus control.

** *P* < 0.01 versus control.

P < 0.01 control versus normal.

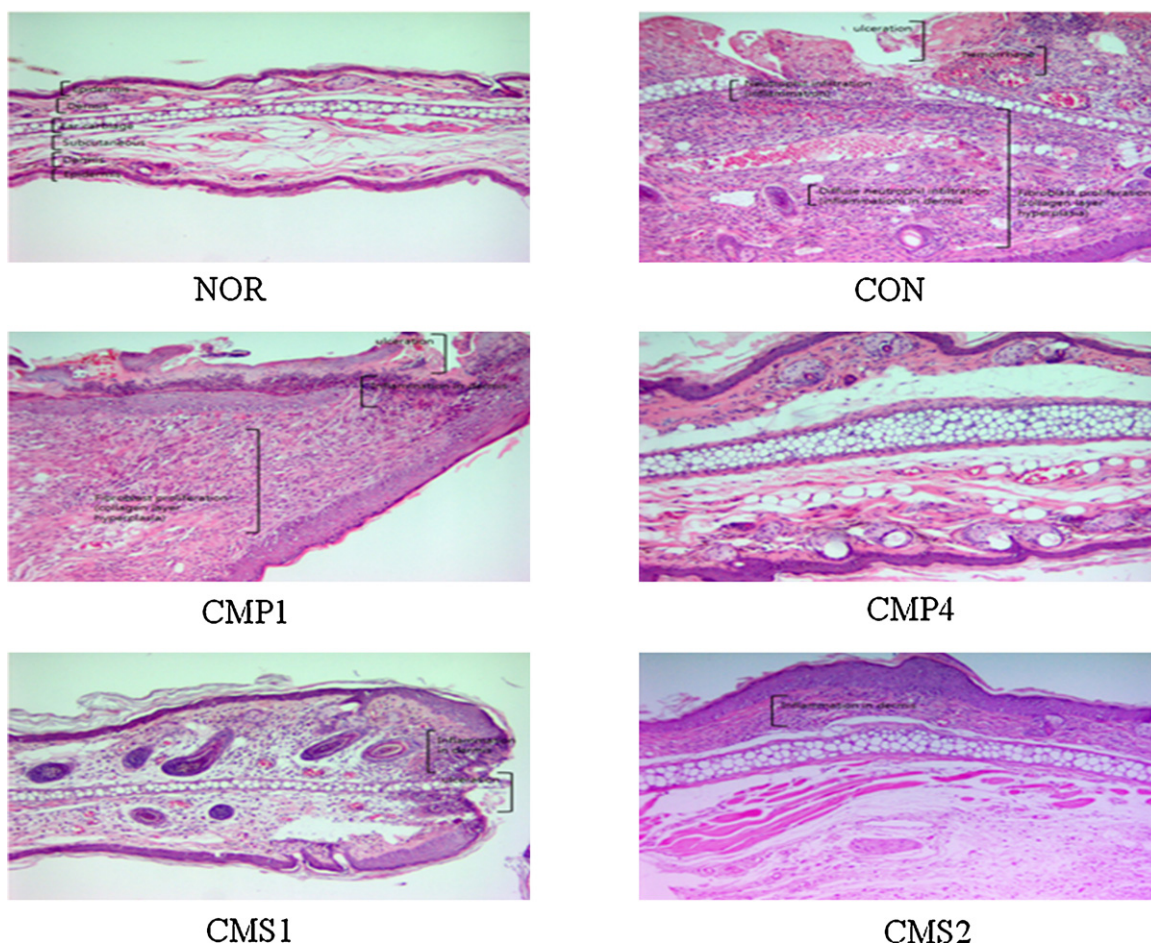


Fig. 2. Histological features of ear skin in the control and in the groups with *Chelidonium Herba*. H&E stain; original magnification 40 $\times$. Dermatitis was elicited by repeated painting of DNCB (in acetone/olive oil) on outer and inner sides of mouse ear. The same volume of acetone/olive oil was painted in control mice. The sections were prepared as described in Section 2, and stained with hematoxylin and eosin.

more biting movements were counted as one episode which ended when the rat returned to the straight-forward position (Maekawa et al., 2002).

2.6. Histopathological examination

Dorsal skin and ear samples of NC/Nga mice were removed, fixed in 10% formalin, embedded in paraffin, and serially sectioned at 4 μ m. Sections were stained with hematoxylin and eosin solution.

2.7. Measurement of serum total IgE, TNF- α , and IL-4 levels

After blood was collected from the mice on the day of sacrifice, serum samples were obtained by centrifugation (1700 $\times$ g, 10 min) and stored at -70°C until use. Total serum IgE levels were measured using an ELISA kit (Shibayagi, Gunma, Japan) according to the manufacturer's instructions. The levels of TNF- α and IL-4 were also measured using a mouse ELISA kit (R&D Systems Inc., Minneapolis, MN, USA).

2.8. Statistics

The differences between the treatment groups were examined using an one-way ANOVA and Student's *t*-test. All data are presented as the mean $\pm$ standard error (S.E.). Significant differences between groups were accepted when the *P*-value was less than 0.05.

3. Results

3.1. CM reduced the clinical severity and itching behavior of AD-like skin lesions induced by DNCB in NC/Nga mice

The AD induced by topical application of DNCB resulted in clinical signs and symptoms of itching, erythema and hemorrhage immediately on the ear and back which was followed by edema, superficial erosion, deep excoriation, scaling and dryness of the skin. These clinical signs were markedly alleviated in CON group. However, CM treatment inhibited the appearance of these skin symptoms (Fig. 1). Using the skin severity score, the skin conditions were evaluated twice a week for 42 days after the first application of DNCB. As shown in Table 1. The dermatitis score of the control group increased rapidly and became significantly different at 7 days as compared to that of the normal group. However, in the CM treated group, the dermatitis score decreased markedly at 14 days compared to that of the control group (Table 1). Itching behaviors in the CM-treated groups were significantly lower than those in the control group (Table 2). These results indicate that CM suppresses the dermatitis spontaneously induced in NC/Nga model mice.

3.2. Effects of ear thickness

In the control group, ear swelling was induced gradually after the 2nd DNCB applications. However, CM treatment significantly inhibited ear swelling (Table 3).

3.3. Effects of CM on serum IgE, TNF- α , and IL-4 levels

The serum levels of total IgE, TNF- α , and IL-4 in NC/Nga mice are shown in table. Application of DNCB for 42 days in the control group considerably enhanced the total serum IgE levels as compared with the normal group, whereas CM treatment significantly reduced IgE levels (Table 4). Similarly, the proinflammatory cytokine, TNF- α , and histamine levels in the control group increased as compared with those in the normal group, whereas the levels in the CM-treated group decreased compared to those in the control group (Table 4). These results demonstrate that treatment with CM prevents the development of dermatitis in NC/Nga mice.

3.4. Histological analysis of the effects of CM and aprepitant in DNCB-induced AD-like skin lesions

Next, H&E staining was performed on specimens from the ears of NC/Nga mice. As shown in (Fig. 2), the histological features in control group indicated epidermal hyperplasia, parakeratosis, hyperkeratosis and dermal edema with a large number of infiltrated mononuclear cells together with some eosinophils. Surprisingly, sections of the ears from CM group showed markedly diminished epidermal hyperplasia and cellular infiltration into the dermis. These histological appearances suggest that CM suppresses the inflammation induced by DNCB. In case of normal group, it showed no signs of inflammation.

4. Discussion

In this study, we first showed that administration of CM significantly reduces ear thickness, clinical signs, and serum IgE levels in DNCB-induced AD-like skin lesions of NC/Nga mouse model. Moreover, CM treatment decreased hyperplasia and hyperkeratosis in the epidermis and infiltration of inflammatory cells into the AD-like skin lesions. These results highlighted protective effects of CM on development of dermatitis in DNCB-applied NC/Nga mice. The topical application of DNCB on the backs of NC/Nga mice was an efficient tool for inducing severe and controlled dermatitis within 6 weeks.

NC/Nga mouse model has been considered as one of the most valuable mouse model representing human AD (Matsuoka et al., 2003; Gao et al., 2004). AD-like skin lesions in NC/Nga mice highly resemble those of human AD that show various grades of signs and symptoms like erythema and hemorrhage, followed by edema, superficial erosion, deep excoriation, alopecia and dryness in the skin. Besides, NC/Nga mice with AD-like skin lesions show IgE hyperproduction in serum, severe itching behavior and invasion of inflammatory cells into the skin (Matsuda et al., 1997; Maekawa et al., 2002; Shiohara et al., 2004). This model has been used for the estimation of drug candidates developed for the treatment of AD. Previously, we established a modified NC/Nga mouse model and suggested that this model might be a useful tool to evaluate drug candidates of AD (Kang et al., 2006). Here, we clearly demonstrated that typical symptoms of AD can be observed in NC/Nga mice by repeated topical application of DNCB. Ear swelling and eczematous skin lesion were observed and plasma level of IL-4 and IgE was markedly increased. Histopathological analysis also showed that infiltration of leukocytes, especially mast cells, into the ear was evident. The production of proinflammatory cytokines, such as TNF- α , by epidermal cells is known as one of the main events mediating the initiation of AD (Homey et al., 2006). TNF- α and other proinflammatory cytokines produced at the initiation stage of AD induce the expression of a variety of chemokines and adhesion molecules which direct the recruitment, proliferation and survival of leukocytes within the skin (Homey et al., 2006). Table 4 shows

that the levels of IL-4 and TNF- α in the serum of the CM group was decreased to a statistically significant extent compared with those in the control group (Table 4). Along with TNF- α which is produced at higher levels in the blood as the age of NC/Nga mice increases, has been known to induce the inflammation reaction by stimulating B cells and activating inflammation. The production of TNF- α in the serum in the CM group was reduced to a statistically significant extent compared with that in the control group (Table 4). However, it was very hard to detect IL-4 in the serum. In our study, CM significantly suppressed DNCB-induced elevation of TNF- α expression in the serum of NC/Nga mice which likely have affected leukocyte recruitment at the site. Kitagaki et al. (1997) reported that Th2-type cytokines, especially IL-4, are predominantly produced by T cells and mast cells in the chronic contact hypersensitivity model.

Itching is a characteristic symptom in various forms of dermatosis, especially atopic dermatitis; consequently, it constitutes major diagnostic criteria (Williams et al., 1994). Scratching their skin with toenails seemed to be the most important factor leading to dermatitis through increasing various immunological responses such as the elevation of serum IgE concentration and number of mast cells (Hashimoto et al., 2004). Therefore effective control of itching is believed to be one of the fundamental approaches in controlling the skin lesions and decreasing various immunological responses. In this study, administration of CM significantly inhibited the itching behavior and decreased the elevation of serum IgE levels in NC/Nga AD model mice.

In conclusion, we demonstrated that the administration of CM inhibited the development of AD-like symptoms in NC/Nga mice. The results from the present study suggest that CM could inhibit the development of AD-like skin lesions in NC/Nga mice through the suppression of Th2 cell response.

Acknowledgement

This work was supported by the Stage of Brain Korea 21 project in Oriental Medical Science Center.

References

- Colombo, M.L., Bosisio, E., 1996. Pharmacological activities of *Chelidonium majus* L. (Papaveraceae). *Pharmacological Research* 33, 127–134.
- Gao, X.K., Fuseda, K., Shibata, T., Tanaka, H., Inagaki, N., Nagai, H., 2005. Kampo medicines for mite antigen-induced allergic dermatitis in NC/Nga mice. *Evidence-Based Complementary and Alternative Medicine* 2, 191–199.
- Gao, X.K., Nakamura, N., Fuseda, K., Tanaka, H., Inagaki, N., Nagai, H., 2004. Establishment of allergic dermatitis in NC/Nga mice as a model for severe atopic dermatitis. *Biological & Pharmaceutical Bulletin* 27, 1376–1381.
- Gilca, M., Gaman, L., Panait, E., Stoian, I., Atanasiu, V., 2010. *Chelidonium majus*—an integrative review: traditional knowledge versus modern findings. *Forschende Komplementärmedizin* 17, 241–248.
- Hashimoto, Y., Arai, I., Nakanishi, Y., Sakurai, T., Nakamura, A., Nakaike, S., 2004. Scratching of their skin by NC/Nga mice leads to development of dermatitis. *Life Sciences* 76, 783–794.
- Homey, B., Steinhoff, M., Ruzicka, T., Leung, D.Y., 2006. Cytokines and chemokines orchestrate atopic skin inflammation. *The Journal of Allergy and Clinical Immunology* 118, 178–189.
- Kang, J.S., Lee, K., Han, S.B., Ahn, J.M., Lee, H., Han, M.H., Yoon, Y.D., Yoon, W.K., Park, S.K., Kim, H.M., 2006. Induction of atopic eczema/dermatitis syndrome-like skin lesions by repeated topical application of a crude extract of *Dermatophagoides pteronyssinus* in NC/Nga mice. *International Immunopharmacology* 6, 1616–1622.
- Kim, H.K., Farnsworth, N.R., Blomster, R.N., Fong, H.H., 1969. Biological and phytochemical evaluation of plants. V. Isolation of two cytotoxic alkaloids from *Chelidonium majus*. *The Journal of Pharmaceutical Sciences* 58, 372–374.
- Kitagaki, H., Ono, N., Hayakawa, K., Kitazawa, T., Watanabe, K., Shiohara, T., 1997. Repeated elicitation of contact hypersensitivity induces a shift in cutaneous cytokine milieu from a T helper cell type 1 to a T helper cell type 2 profile. *The Journal of Immunology* 159, 2484–2491.
- Kupeli, E., Kosar, M., Yesilada, E., Husnu, K., Baser, C., 2002. A comparative study on the anti-inflammatory, antinociceptive and antipyretic effects of isoquinoline alkaloids from the roots of Turkish Berberis species. *Life Sciences* 72, 645–657.
- Lenfeld, J., Kroutil, M., Marsalek, E., Slavik, J., Preininger, V., Simanek, V., 1981. Antiinflammatory activity of quaternary benzophenanthridine alkaloids from *Chelidonium majus*. *Planta Medica* 43, 161–165.

- Leung, D.Y., 2000. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *The Journal of Allergy and Clinical Immunology* 105, 860–876.
- Maekawa, T., Yamaguchi-Miyamoto, T., Nojima, H., Kuraishi, Y., 2002. Effects of naltrexone on spontaneous itch-associated responses in NC mice with chronic dermatitis. *The Japanese Journal of Pharmacology* 90, 193–196.
- Matsuda, H., Watanabe, N., Geba, G.P., Sperl, J., Tsudzuki, M., Hiroi, J., Matsumoto, M., Ushio, H., Saito, S., Askenase, P.W., Ra, C., 1997. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *International Immunology* 9, 461–466.
- Matsuoka, H., Maki, N., Yoshida, S., Arai, M., Wang, J., Oikawa, Y., Ikeda, T., Hirota, N., Nakagawa, H., Ishii, A., 2003. A mouse model of the atopic eczema/dermatitis syndrome by repeated application of a crude extract of house-dust mite *Dermatophagoides farinae*. *Allergy* 58, 139–145.
- Pugliarello, S., Cozzi, A., Gisondi, P., Girolomoni, G., 2010. Phenotypes of atopic dermatitis. *Journal der Deutschen Dermatologischen Gesellschaft* 9, 12–20.
- Saglam, H., Arar, G., 2003. Cytotoxic activity and quality control determinations on *Chelidonium majus*. *Fitoterapia* 74, 127–129.
- Shiohara, T., Hayakawa, J., Mizukawa, Y., 2004. Animal models for atopic dermatitis: are they relevant to human disease? *The Journal of Dermatological Science* 36, 1–9.
- Tin-Wa, M., Kim, H.K., Fong, H.H., Farnsworth, N.R., 1972. The structure of chelidimerine, a new alkaloid from *Chelidonium majus*. *Lloydia* 35, 87–89.
- Williams, H.C., Burney, P.G., Pembroke, A.C., Hay, R.J., 1994. The U.K Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. *The British Journal of Dermatology* 131, 406–416.