

# Anti-Allergic Effect of *Ulmus davidiana* Cortex on Contact Dermatitis Induced by Dinitrofluoro-Benzene in Mice

Jeonghyeon Lyu<sup>1</sup>, Byung-Joo Kim<sup>2</sup>, Hyungwoo Kim<sup>1\*</sup>

<sup>1</sup> Division of Pharmacology, Pusan National University School of Korean Medicine, Yangsan, Korea

<sup>2</sup> Division of Longevity and Biofunctional Medicine, Pusan National University School of Korean Medicine, Yangsan, Korea

## Key Words

allergy, dermatitis, inflammation, skin, *Ulmus davidiana*

## Abstract

**Objective:** The root bark of *Ulmus davidiana* var. Japonica (Ulmi Radicis cortex, URC) is a medicinal herb used for promoting diuresis and treating dampness. In Korea, URC has long been used as an efficacious therapy for inflammation, burns, frostbite and skin diseases such as eczema and psoriasis.

**Methods:** In the present study, we used 1-fluoro-2,4-dinitrofluorobenzene (DNFB)-induced contact dermatitis (CD) mouse model to investigate the anti-allergic and the anti-inflammatory effects of URC on skin lesion, histopathological changes and specific antibody production.

**Results:** URC treatment, 10 mg/mL, effectively inhibited skin lesions induced by repeated paintings with DNFB. In the histopathological observation, topical application of URC inhibited spongiosis. In addition, URC lowered the production levels of total immunoglobulin and IgG2a in serum.

**Conclusion:** These data indicate that URC has an anti-

inflammatory effect that produces an improvement of skin lesions in CD mice.

## 1. Introduction

In Korea, the root bark of *Ulmus davidiana* var. Japonica (Ulmi Radicis cortex, URC) is widely used to treat patients with allergic skin diseases such as eczema, pruritus and urticaria. In the theory of traditional medicine, URC can be used to promote diuresis and to treat dampness [1]. Previous studies have shown that URC or its isolated components have various effects, including anti-oxidation [2, 3], anti-inflammatory [4], and possibly cosmetic effects [5].

Contact dermatitis (CD) has an important economic and occupational health impact on society. CD presents as an inflammatory response to specific agents such as nickel and involves both skin resident cells and activated immune cells [6]. A widely used animal model of human CD is the delayed type hypersensitivity response to haptens such as dinitrofluorobenzene (DNFB) and dinitrochlorobenzene (DNCB) in mice [7]. Repeated applications of DNFB or DNCB are well known to induce typical features of CD, such as swelling, erythema and scale in the inflamed area [8].

Based on this background, we investigated the effects of URC by using a mouse model of CD. In the present

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\* Corresponding Author

Hyungwoo Kim, Division of Pharmacology, Pusan National University School of Korean Medicine, Beomeo-ri, Mulgeum-eup, Yangsan, Gyeongnam 626-870, Korea.

Tel: +82-51-510-8458 Fax: +82-51-510-8420

E-mail: kronos7@pusan.ac.kr

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study, we investigated the effects of URC on skin lesions, histopathological changes of tissues, and levels of antibodies in serum.

## 2. Materials and methods

### 2.1. Chemicals and reagents

(DNFB) 1-fluoro-2,4-dinitrofluorobenzene, olive oil, and goat anti-mouse polyvalent antibody were purchased from Sigma-Aldrich (St. Louis, MO, USA). Goat anti-mouse polyvalent antibody, goat anti-mouse IgG1 antibody and goat anti-mouse IgG2a antibody were purchased from Southern Biotech (Birmingham, AL, USA).

### 2.2. Preparation of URC

Fifty g of URC were extracted using 99.9% methanol for 24 h. The extract was filtered and evaporated under reduced pressure by using a vacuum evaporator (Eyela, Japan). The condensed extract was then lyophilized. Finally, 3.57 g of lyophilized powder was obtained (yield; 7.1%).

### 2.3. Animals

Male balb/c mice (6 week old) were purchased from Samtaco (Incheon, Korea). The mice were housed under specific pathogen-free conditions with a 12 h light/dark cycle and free access to standard rodent food and water. All animal experiments were approved by our Animal Care and Use Committee and were performed according to institutional guidelines (PNU-2011-000406).

### 2.4. Induction of CD and experimental design

Mice were sensitized by painting with 30  $\mu$ l of DNFB (0.1%, v/v) in acetone:olive oil (AOO, 4:1) on the dorsum of each ear for three consecutive days. Three days after sensitization, the dorsa of the mice were shaved. Four days after sensitization, the mice were challenged by painting the shaved dorsa with 50  $\mu$ l of DNFB (0.2%, v/v) in AOO every two days. URC was dissolved in ethanol, was then filtered using a syringe filter (0.45  $\mu$ m), and was finally diluted in AOO (ethanol:AOO, 4:1). Ten mg/mL of URC solution was painted on the shaved dorsa for 6 days. Naive animals (Naive) were treated with the vehicle and was painted with the vehicle (n = 6). Control animals (CTL) were sensitized and challenged with DNFB and then painted with the vehicle (n = 8). URC-treated animals were sensitized and challenged with DNFB and then painted with 10 mg/mL of URC solution (n = 8). The experimental design is shown in Fig. 1.

### 2.5. Measurement the degree of skin lesion

At the end of experiment, in order to observe the overall degree of CD, we sacrificed the mice and observed the skins

of the dorsa by using a digital camera (Olympus, Japan).

### 2.6. Histopathological examination

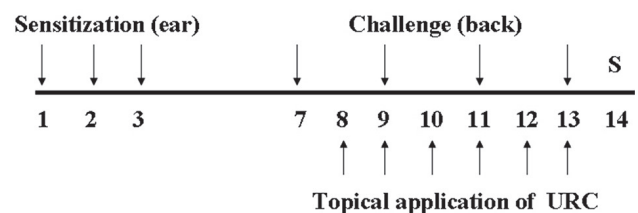
After measurements of the thicknesses and the weights of the ears, ear tissues were resected and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) for histopathological observations such as immune cell infiltration and spongiosis. Stained tissues were observed using a light microscope (x100).

### 2.7. Measurement of immunoglobulin production

The levels of total antibody, IgG1 and IgG2a in serum were measured by using an enzyme-linked immunosorbent assay (ELISA). Plates with 96 well (Nunc, USA) were coated with goat anti-mouse polyvalent antibody (Sigma-Aldrich, 1:1000) at RT for 3 h and incubated at 4  $^{\circ}$ C overnight. After the plate had been washed, 100  $\mu$ l of diluted serum (1:20 in phosphate-buffered saline, PBS) was added to each well, and the plates were incubated at RT for 2 h. After the plate had been washed again, alkaline phosphatase-conjugated anti-mouse immunoglobulin was added, and the plates were incubated at RT for 1 h. After incubation, para-nitrophenyl phosphate (p-NPP) was added. The optical density (OD) was measured at 405 nm by using a microplate spectrophotometer (BioTek, VT, USA). Secondary antibodies used were goat anti-mouse polyvalent antibody (1:1000), goat anti-mouse IgG1 antibody (1:1000) and goat anti-mouse IgG2a antibody (1:1000). The serum obtained from another experiment was used as a standard serum and all the titers were calculated as values relative to that serum.

### 2.8. Statistical analysis

All statistical comparisons were made with the Student's *t*-test. The SigmaPlot version 11.0 (SYSTAT software, USA) was used for the statistical analysis. All data were represented as mean  $\pm$  SD. Differences with values of *P* < 0.05 were considered as significant.



**Figure 1** Experimental design. The experimental groups, except the naive group, were sensitized by painting with DNFB on days 1, 2 and 3. Then, they were challenged on days 7, 9, 11, and 13. The naive group was treated with AOO in the same way. The URC group was painted with URC on days 8, 9, 10, 11, 12, and 13. All animals were sacrificed on day 14.

### 3. Results

#### 3.1. Effects of URC on the degree of skin lesions in CD mice

URC improved skin lesions in CD mice. Repeated painting with DNFB resulted in skin lesions of CD such as erythema, scaling and crust (Fig. 2B). The group treated with 10 mg/mL of URC showed diminished skin lesions compared to the vehicle-treated control group (Fig. 2C).

#### 3.2. Effects of URC on histopathological changes in CD mice

No abnormal changes in skin tissues were observed in the naive group (Fig. 3A). The epidermis in the control group showed hyperplasia, and significant edema and spongiosis. In addition, marked infiltrations of mononuclear cells were observed in the dermis (Fig. 3B). The URC-treated group showed diminished hyperplasia, edema and spongiosis compared to the non treated control group (Fig. 3C).

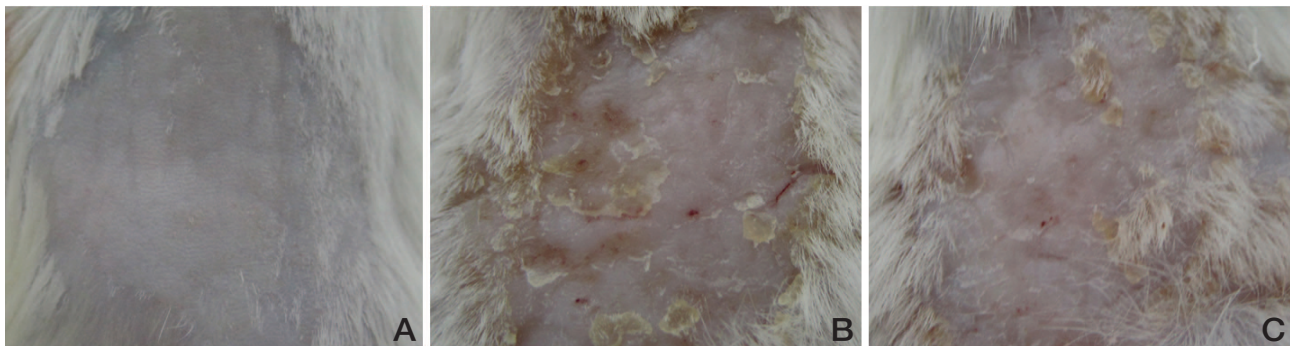
#### 3.3. Effects of URC on production levels of antibodies in CD mice

URC lowered the production levels of total antibodies and IgG2a in serum. Repeated applications of DNFB resulted in increased levels of total antibodies and IgG2a in serum. In our model, the level of IgG1 in the control group was almost the

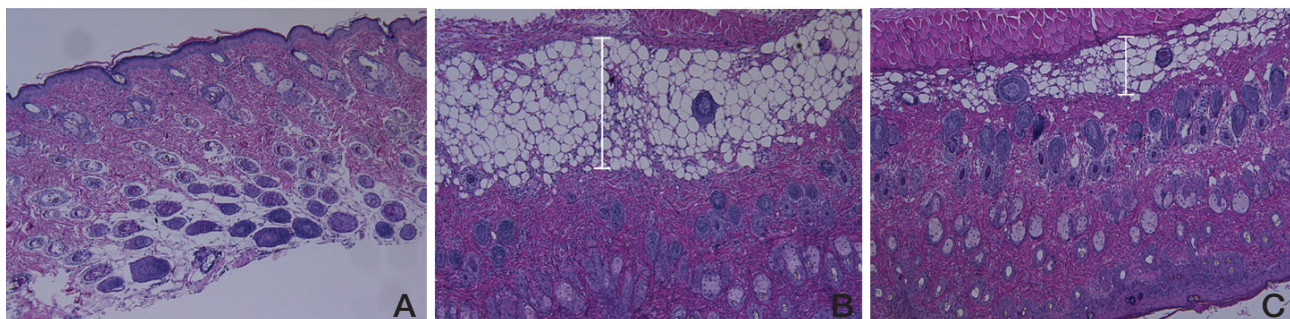
same as that in the naive group. Treatment with 10 mg/mL of URC significantly suppressed the increases in the total antibody and the IgG2a levels (Fig. 4A, C). The level of IgG2a was not affected by topical application of URC (Fig. 4B).

### 4. Discussion

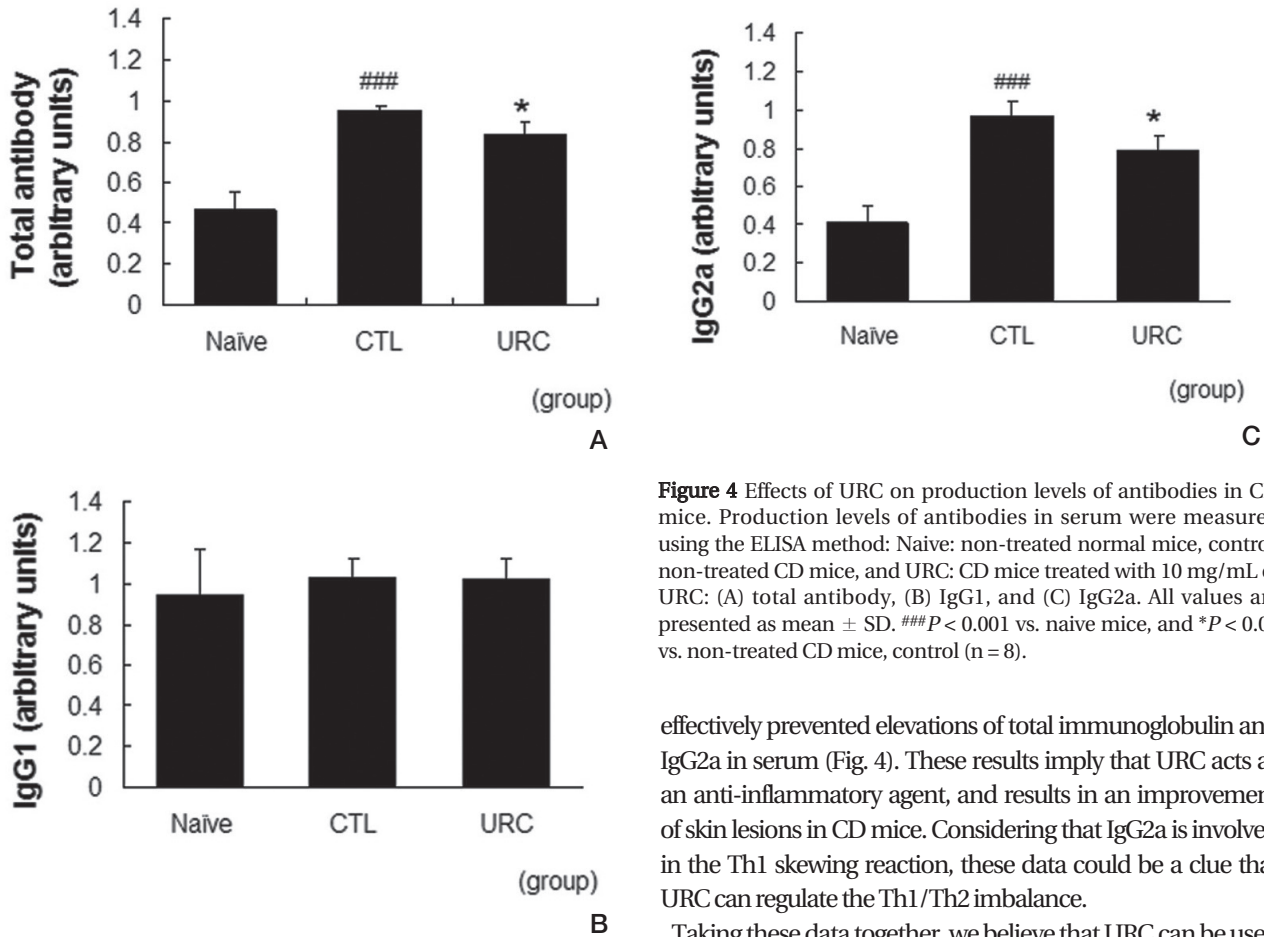
Occupational skin diseases are the most common work-related disorders worldwide. The most common cause of occupational skin diseases is CD [9]. The main etiologic treatment of CD is avoidance of the contact allergen, but in some cases, especially in occupational diseases, elimination of the offending agent is impossible. For this reason, therapy such as the use of corticosteroids, is rendered to assuage the inflammatory component [10, 11]. Because corticosteroids have some adverse effects, their usages have been restricted, so many of scientists are investigating complementary medicine and alternatives to corticosteroids. Recently, some research has focused on the use of medicinal plants as complementary medicine and as alternatives to corticosteroids, possibly because their low cost and safety [12]. In Korea, many medicinal herbs such as *Sophora flavescens* Aiton [13], *Scrophularia buergeriana* [14], *Dictamnus dasycarpus* Turcz [15] and URC have long been



**Figure 2** Effects of URC on skin lesions in CD mice. Skin conditions were observed using a digital camera: (A) Naive mice, (B) CD-induced control (CTL) mice, and (C) URC-treated CD mice.



**Figure 3** Effects of URC on histopathological changes in CD mice. Tissues were stained with hematoxylin and eosin and were observed using a light microscope: (A) naive mice, (B) CD-induced control (CTL) mice, and (C) URC-treated CD mice. Filled bars indicate areas of spongiosis (x100).



**Figure 4** Effects of URC on production levels of antibodies in CD mice. Production levels of antibodies in serum were measured using the ELISA method: Naive: non-treated normal mice, control: non-treated CD mice, and URC: CD mice treated with 10 mg/mL of URC: (A) total antibody, (B) IgG1, and (C) IgG2a. All values are presented as mean  $\pm$  SD. ### $P$  < 0.001 vs. naive mice, and \* $P$  < 0.05 vs. non-treated CD mice, control (n = 8).

effectively prevented elevations of total immunoglobulin and IgG2a in serum (Fig. 4). These results imply that URC acts as an anti-inflammatory agent, and results in an improvement of skin lesions in CD mice. Considering that IgG2a is involved in the Th1 skewing reaction, these data could be a clue that URC can regulate the Th1/Th2 imbalance.

Taking these data together, we believe that URC can be used to treat patients with CD and may be useful as a complementary medicine or as an alternative to corticosteroids.

## 5. Conclusion

This study was designed to investigate the anti-allergic effects of URC by using a DNFB induced CD mouse model. We showed that URC could reduce skin lesions in inflamed tissues, epidermal spongiosis, and edema in histopathological observations. We also showed that URC could lower the production levels total antibodies and IgG2a in serum. These data suggest that URC can be used to treat patients with allergic skin diseases and that related mechanisms are involved in the anti-inflammatory action on the Th 1 skewing reaction.

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used for the treatment of dermatological disorders.

In the present study, we showed the anti-allergic action of URC by using a mouse model of CD. Topical application of URC suppressed allergic reactions in the DNFB-induced CD model for mice. Skin lesions, such as erythema, crust and scaling, were diminished by topical application of URC (Fig. 2). Epidermal hyperplasia, which was related to inflammatory reactions with resident cells such as fibroblasts and keratinocytes, was slightly decreased in the histopathological observations (Fig. 3). In addition, epidermal edema and spongiosis were diminished by topical application of URC (Fig. 3). Intercellular epidermal edema is well known to be able to induce spongiosis, and is recognized as the microscopic hallmark of an inflammatory skin disease such as CD [16]. These data imply that epidermal spongiosis, edema and inflammatory cell infiltration result in skin lesions such as erythema, scaling and crust, and that the use of URC effectively prevented these inflammatory reactions in DNFB-induced CD.

Specific antibodies are well known to be involved in the pathophysiology of CD. In our results, repeated paintings with DNFB elevated the serum levels of total immunoglobulin and IgG2a. Topical application of URC

and Technology (2012011676). The authors declare that there are no conflicts of interest.

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