Therapeutic effects of shikonin on skin lesions in mouse models of allergic dermatitis and wound

Keiichi Kadoyama,1 Mariko Takenokuchi,1 Kenji Matsuura,1 Hiroaki Shichiri,1 Aimi Watanabe,1 Hirotsugu Yamaguchi,2 Hisashi Takahashi,3 Hiromi Takano-Ohmuro4 & Taizo Taniguchi1,5*

1 Department of Pharmaceutical Health Care, Faculty of Pharmaceutical Sciences, Himeji Dokkyo University, Himeji, Japan
2 Institute of Pathology, Kyodo Byori Inc., Kobe, Japan
3 Biostir Inc., Osaka, Japan
4 Research Institute of Pharmaceutical Sciences, Musashino University, Nishitokyo, Japan
5 Pharma Crea Kobe Co., Ltd., Kobe, Japan

ABSTRACT

Aim: The aim of this study was to examine the beneficial effects of shikonin on skin lesions in mouse models of allergic dermatitis and wound.

Methods: Mouse models of allergic dermatitis and wound were generated by spreading an allergic-inducing reagent on the dorsal skin and ear flaps, and by punching the dorsal skin, respectively. The effects of shikonin on allergic dermatitis was evaluated on frequency of scratching, clinical severity of dermatitis and immunohistochemistry. The effect of shikonin on wound healing compared with steroid was evaluated by the size of the wound area.

Results: Shikonin treatment not only improved allergic dermatitis, but also prevented the onset of allergic dermatitis. Furthermore, shikonin promoted wound healing in a mouse model of skin wound. In contrast, steroid (betamethasone) treatment, which is often applied to treat allergic dermatitis, delayed wound healing in the mouse model of allergic dermatitis.

Conclusion: Shikonin can be used as a new therapeutic agent for the treatment of allergic dermatitis including atopic dermatitis.

KEY WORDS: skin disease, traditional herbal medicine, wound healing

INTRODUCTION

Shikonin is a naphthoquinone compound extracted from the root of Lithospermum erythrorhizon (LE). It has long been used as an oriental traditional medicine ointment for wound healing. Shikonin has been shown to have various biological activities such as inhibition of tumor progression, antibacterial effects, acceleration of wound healing, and anti-inflammatory activities both in vitro and in vivo [1–3]. Shikonin also inhibits the expression of tumor necrosis factor-α (TNF-α) and lipid peroxidation, which contribute to the pathogenesis of both acute and chronic inflammatory diseases [4–6]. Recently, we have reported that shikonin inhibits histamine release from human basophils through inhibition of spleen tyrosine kinase (Syk)-dependent phosphorylation [7], and directly inhibits nitric oxide (NO) production via inhibiting NO synthase induced by lipopolysaccharide in murine RAW 264.7 macrophages [8]. Furthermore, shikonin decreases mRNA levels of key gene candidates involved in inflammation, such as CYBA (component of the superoxide-generating Nox2 enzyme), GSK3B (controller of cell responses after toll-like receptor stimulation), and EIF4E, a key factor of the eukaryotic translation initiation factor 4F complex that regulates the abundance of other proteins involved in immune functions [9]. These data suggest that shikonin would be beneficial for treatment of allergic skin diseases including atopic dermatitis (AD).

Atopic dermatitis is one of the major pruritic inflammatory skin diseases; it has a strong genetic component and is characterized by hypersensitivity against various types of antigens [10]. This disease is characterized by poorly defined erythema with edema, vesicles, and weeping in the acute stage, and by skin thickening in the chronic stage [11]. With the increasing prevalence of AD [12], numerous treatment approaches have been devised and are still promoted. They are divided into three groups: anti-inflammatory drugs, skin-
barrier reconstructing creams; and physical approaches. Of these, steroidal anti-inflammatory drugs, such as betamethasone, are the most often used to treat AD because they have a very strong anti-inflammatory action through inhibiting several immune responses. The risk of prolonged use of steroids, however, has been demonstrated in several studies [13]. For example, steroids inhibit angiogenesis and increase of collagen fiber at the scratching site, leading to delayed wound healing [14]. These actions would be disadvantageous in AD therapy with steroids.

In this study, we therefore examined (i) the therapeutic and preventive effects of shikonin on allergic skin diseases using a mouse model of AD; and (ii) the wound healing effects of shikonin on skin wound to determine whether shikonin is an effective medicine alternative to steroids for allergic dermatitis.

METHODS

Animals

Male Nishiki-nezumi Cinnamon/Nagoya (NC/Nga) mice aged 10 weeks (obtained from Charles River Laboratories International, Yokohama, Japan) and male C57BL/6 mice (obtained from Japan SLC, Shizuoka, Japan) aged 7 weeks were used as mouse models of allergic dermatitis [15] and skin wound, respectively. NC/Nga mice raised in non-air-controlled conventional circumstances spontaneously develop allergic dermatitis-like skin lesions [16]. Mice were housed under standard illumination parameters (12 h light/12 h dark cycle; light on at 07:00 hours) and were given free access to standard chow and water. We performed all animal experiments in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the experimental protocol was approved by the Biostir Animal Experiment Committee and the Himeji Dokkyo University Animal Experiment Committee.

Effects of shikonin on allergic dermatitis

Induction of allergic dermatitis

Allergic dermatitis was induced by spreading an allergic-inducing reagent, Biostir AD (Biostir, Osaka, Japan), which is a natural chemical made from allergens of Dermatophagoides farinae according to Yamamoto et al. [17]. Briefly, on induction (day 0), we removed the hair on the dorsal skin and earflap region of male NC/Nga mice aged 10 weeks using a depilatory cream (Krace, Tokyo, Japan). After depilation, a 4% sodium dodecyl sulfate (Wako Pure Chemical Industries, Osaka, Japan) aqueous solution (150 μL) was spread on the dorsal skin and earflaps and was allowed to dry for 2–3 h to impede fat-forming element removal and damage the cuticle barrier. Subsequently, an allergic-inducing reagent (Biostir AD ointment 100 mg) was spread on the dorsal skin and earflaps using a flat stick. These procedures were repeated twice per week until the last application on day 17.

Evaluation of skin lesions

The clinical severity of dermatitis on the dorsal skin and earflap was scored using a previously described macroscopic diagnostic scoring system [17]. Briefly, the development of each of the following conditions (i) erythema/hemorrhage; (ii) scarring/dryness; (iii) edema; and (iv) excoriation/erosion, was scored as 0, none; 1, mild; 2, moderate; or 3, severe. The sum of the individual scores (the dorsal skin and earflap) was taken as the dermatitis score, ranging from a minimum of 0 to a maximum of 12 points.

Evaluation of allergic dermatitis treatment

On day 21, dermatitis score was evaluated, and mice with a dermatitis score 6–9 were used for therapeutic examination as a mouse model of allergic dermatitis. The allergic dermatitis model mice were divided into two equivalent groups, with an equal mean dermatitis score of 8 points. A total of 100 mg Vaseline ointment with 10 μmol/L shikonin (Wako Pure Chemical Industries; shikonin group) or without shikonin (control group) was applied to the dorsal skin and earflaps daily, and dermatitis score was evaluated and compared between the two groups.

Behavioral observation of scratching

The mice were placed in an acrylic cage, and their nocturnal scratching behavior was recorded under unmanned conditions using a video camera (Canon, Tokyo, Japan) with an infrared lamp and a hard disc recorder, from 18:00 hours to 02:00 hours. Given that the mice generally scratched for approximately 1 s, then 1 s of scratching was counted as one incident of scratching, and the number of scratches of the dorsal skin and earflap by the hind paws was counted.

Prevention of allergic dermatitis onset

Fifteen NC/Nga mice aged 10 weeks were used. Treatment with Biostir AD ointment (100 mg) was performed in the same way to induce allergic dermatitis, for 3 weeks. Shikonin was applied on days without Biostir AD treatment. Dermatitis score was evaluated and compared with that of nontreated groups on days 0, 7, 14, and 21.

Effects of shikonin on wound healing

Wound model

C57BL/6 mice aged 7 weeks were used. Under i.p. injection of pentobarbital sodium anesthesia, the dorsal skin was shaved and sanitized with 70% (w/w) ethanol, and two full-thickness excisional wounds were generated (wounded day 0) with a sterile 3 mm biopsy punch (FEATHER, Tokyo, Japan). A total of 100 mg Vaseline ointment with 10 μmol/L shikonin (shikonin group), 0.12% betamethasone valerate (Rinderon-V ointment, Shionogi, Osaka, Japan; steroid
group), or Vaseline (White Petrolatum, Kenei Pharmaceutical, Osaka, Japan; control group), was applied to the dorsal skin daily. Two wounds were measured daily and each lesion area was calculated.

**Immunohistochemistry**

To observe the change in skin tissue, the mice were killed under i.p. injection of pentobarbital sodium anesthesia on wounded day 4 after 3 days of treatment. The skin including the wounded site was isolated and fixed in 10% neutral buffered formalin solution overnight. Subsequently, the skin tissues were embedded in paraffin. Serial tissue sections were cut on a run-type microtome (TU-213; Yamato-Kohki, Saitama, Japan) to a thickness of 3 μm, deparaffinized, and used for either hematoxylin–eosin (HE) staining or for immunohistochemistry. On immunohistochemistry the sections were stained with antibodies against interleukin-1β (IL-1β; 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and basic fibroblast growth factor (bFGF; 1:500; Santa Cruz Biotechnology). After deparaffinization, the slides were heated using an autoclave at 121°C for 5 min in 0.01 mol/L citrate buffer at pH 6.0 for antigen retrieval. Immunohistochemistry was performed on a sample following paraffin embedding. Subsequently, the slides were treated with 0.3% hydrogen peroxide for 30 min to inactivate endogenous peroxidase activity and were stained with horseradish peroxidase (HRP). The sections were then blocked with Blocking One (Nacalai Tesque, Kyoto, Japan) at room temperature for 10 min. IL-1β or bFGF was stained with corresponding antibody followed by biotin-labeled secondary antibody (Dako Cytomation, Glostrup, Denmark), HRP-labeled streptavidin (Dako Cytomation), and the substrate diaminobenzidine. All sections were analyzed, and images were obtained using a microscope (Olympus, Tokyo, Japan) equipped with a digital camera. The digital images were adjusted to an appropriate figure size required but were otherwise not processed.

**Statistical analysis**

All data are shown as mean ± SEM. Student’s t-test or Wilcoxon signed-rank test was performed to evaluate the differences between groups using Ekuseru-Toukei 2015 (Social Survey Research Information, Tokyo, Japan). P < 0.05 was considered statistically significant.

**RESULTS**

**Shikonin reduces scratching in a mouse model of allergic dermatitis**

Shikonin has been shown to possesses many biological activities through various pharmacological mechanisms [1–3]. Of these, we previously reported that shikonin inhibits immunoglobulin (IgE)-mediated histamine release, suggesting that shikonin may improve allergic disorders [7]. We initially focused on allergic dermatitis. To evaluate the beneficial effects of shikonin, we generated a mouse model of allergic dermatitis following the methods described by Yamamoto et al. [17]. We examined whether shikonin reduces scratching in allergic dermatitis model mice, which is one of the characteristic behavioral symptoms of allergy. Twelve mice with total dermatitis score >6 were used, and the mice were divided into two identical groups. One group was treated with Vaseline ointment containing 10 μmol/L shikonin (shikonin group), and the other was treated with an ointment only (control group). One mouse belonging to the control group died during the examination, and its data were omitted from the result. Macroscopic observation of allergic dermatitis in a mouse model is shown in Figure 1a. After 7 days of treatment, time spent in scratching in the shikonin group reduced to 60% of day 0, but that in the control group showed little change (Fig. 1b). After 14 days of treatment, time spent in scratching in the shikonin group reduced to nearly 40% of that on day 0, but in the control group, it still remained above 80% of that on day 0 (Fig. 1b).

After these observations, all mice were evaluated by checking the presence or absence of pathology findings, such as erosion, inflammation, hyperplasia, hemorrhage, collagen fiber, and fibroblast, in the dorsal skin and ear flap of the control (n = 5) and shikonin groups (n = 6), and calculating the odds ratio for each finding at day 14 (Table 1). The pooled risk difference (DerSimonian–Laird method) for each pathology finding was 0.3035. The 95% CI of the pooled risk difference was 0.1429–0.4641 (P = 0.0002; Table 1). This suggests that shikonin has beneficial effects on allergic dermatitis.

**Shikonin improves skin lesion in a mouse model of allergic dermatitis**

We examined if shikonin improved the skin lesion of allergic dermatitis. Thirty mice with total dermatitis score >6 were obtained and divided into two identical groups. Macroscopic features of allergic dermatitis-like symptoms, such as erythema/hemorrhage, scarring/dryness on the dorsal skin edema, and excoriation/erosion on the ear flap, were observed and scored as dermatitis score. As shown in Figure 1c, shikonin treatment improved macroscopic features both on the dorsal skin and ear flap. The dermatitis score in the control group from treatment days 0 to 14 decreased from (mean ± SEM) 3.5 ± 0.22 to 2.5 ± 0.43 on the dorsal skin, from 4.0 ± 0.26 to 3.7 ± 0.21 on the ear flap, and, in total, from 7.3 ± 0.47 to 6.2 ± 0.48. The dermatitis score in the shikonin group from treatment days 0 to 14 decreased from 3.3 ± 0.21 to 1.5 ± 0.50 on the dorsal skin, from 4.0 ± 0.26 to 2.8 ± 0.60 on the ear flap, and, in total, from 7.3 ± 0.33 to 4.3 ± 0.95. The total score in the shikonin group (dorsal skin and ear flap) reduced significantly faster than that in the control group (Fig. 1c; n = 15, P < 0.05). These results also support the therapeutic effects of shikonin.
Shikonin prevents the onset of allergic dermatitis

The data in the previous section all related to treatment after the condition had been established. Next, we focused on prevention. Thirty mice were treated with an allergic-inducing reagent, Biostir AD ointment (100 mg). Shikonin was used in half (shikonin group) and not in the other half (control group) during AD induction. The dermatitis scores were evaluated on days 0, 7, 14, and 21. As shown in Figure 1d, on days 7, 14, and 21, the dermatitis scores of the control group were 1.5 ± 0.22, 2.4 ± 0.32, and 2.7 ± 0.23 on the dorsal skin; 1.5 ± 0.26, 2.9 ± 0.32, and 3.8 ± 0.22 on the earlap; and 3.1 ± 0.43, 5.3 ± 0.61, and 6.5 ± 0.38 for the total, respectively. The dermatitis scores of the shikonin group on days 7, 14, and 21 were 0.80 ± 0.11, 1.9 ± 0.23, and 2.0 ± 0.22 on the dorsal skin; 1.5 ± 0.26, 2.9 ± 0.32, and 3.8 ± 0.22 on the earlap; and 1.9 ± 0.34, 4.5 ± 0.43, and 4.8 ± 0.55 for the total, respectively. The scores of the shikonin group at days 7 and 21 were significantly reduced (n = 15, P < 0.05) compared with the control group (Fig. 1d). This suggests that shikonin has preventive and therapeutic actions for allergic dermatitis.

Shikonin but not steroids promotes wound healing

In the clinical field, steroids, such as betamethasone and dexamethasone, are often used for allergic dermatitis including AD. Steroids have an anti-inflammatory action via the
inhibition of infiltration of the inflammatory cells [14]. Although steroids are very effective, their adverse effects, especially on wound healing, have been reported in many studies [18]. Steroids delay wound healing by inhibiting angiogenesis and increase of collagen fiber, and suppressing mRNA expression of keratinocyte growth factor, which plays...
an important function in the morphogenesis of epithelium and wound re-epithelialization at the wound site [14,19,20]. In contrast, shikonin promotes wound healing in skin [21–23]. Shikonin also stimulates the migration of intestinal epithelial cells through a transforming growth factor (TGF)-β-dependent process, which could promote the healing of an intestinal injury [24]. To compare the effects of shikonin and steroids on wound healing, we generated a wound mouse model by punching the dorsal skin. During the experimental period, bodyweight loss in wounded model mice was not observed (data not shown). A dose of 10 μmol/L shikonin (shikonin group), 0.12% betamethasone (steroid group), or Vaseline (control group) was applied on the dorsal skin of the wounded mice. As shown in Figure 2a,b, the macroscopic wound size from day 3 to day 6 in the shikonin group was smaller than that in the control group. In contrast, the wound size from day 4 to day 5 in the steroid group was bigger than that in the control group. On measurement of the wounded area in the three groups, it was decreased immediately from wounded day 2 in the shikonin group as compared with the steroid group. These results are consistent with the reports that steroids delay wound healing, and with those reporting that shikonin promotes wound healing in skin.

Histopathological changes usually precede macroscopic changes. Therefore, the mice on day 3 were analyzed on histochernistry. Acute inflamations, such as erosion, edema, and exudate, were mild in the shikonin and steroid groups compared with those in the control group (Fig. 3a). Skin regeneration, including hyperplasia of fibroblast and epidermal cells, was observed in the shikonin group (Fig. 3a). In addition, on immunohistochemistry, IL-1β, which is an important mediator of the inflammatory response, was detected in all groups, and IL-1β immunoreactivity (IR) in the shikonin and steroid groups was weaker than that in the control group (Fig. 3b). Furthermore, bFGF, which possesses diverse biological activities, such as wound healing, was also detected in all groups, and the intensity of bFGF-IR in the shikonin group was stronger and broader than that in other groups (Fig. 3b). This suggests that shikonin inhibited inflammatory reaction through downregulation of IL-1β expression and promoted wound healing through upregulation of bFGF expression at the wound skin site.

**DISCUSSION**

The aim of this study was to determine whether shikonin is an effective drug alternative to steroids for allergic dermatitis. For this purpose, we examined the therapeutic and preventive effects of shikonin on allergic skin disease and wound healing, using mice models of allergic dermatitis and skin wound. We first showed that shikonin significantly decreased scratching (Fig. 1b) and improved the clinical severity of allergic dermatitis (Fig. 1a,c) through inhibition of inflammation (Table 1). Second, shikonin significantly reduced the clinical severity of allergic dermatitis (Fig. 1d). Third, shikonin promoted wound healing compared with

![Figure 3](https://example.com/figure3.png)

**Figure 3** | Histological changes of the dorsal skin in the wound model mice treated with shikonin or betamethasone (steroid). Control, Vaseline. (a) Hematoxylin–eosin (HE)-stained wound sections. Scale bar, 200 μm. (b) Immunohistochemical interleukin (IL)-1β- and basic fibroblast growth factor (bFGF)-stained wound sections. Arrowheads, immunoreactivity for IL-1β or bFGF.
steroid and control (Fig. 2), likely through the inhibition of IL-1β expression and enhancement of bFGF expression (Fig. 3). In contrast, steroids delayed wound healing compared with the control (Fig. 2). Based on these findings, shikonin may be a new therapeutic agent for the treatment of allergic dermatitis.

The first drug choice among topical treatments for allergic dermatitis, especially AD, is corticosteroids (steroids). Although the strong anti-allergic and anti-inflammatory effects of steroids could treat allergic dermatitis immediately, prolonged steroid application has potential adverse effects [13,25]. For example, steroid-induced skin atrophy is the most frequent, and perhaps most important, cutaneous side-effect of long-term topical steroid therapy [25,26]. This side-effect is mainly caused by suppressive effects on cutaneous cell proliferation and protein synthesis [24]. In addition, steroids delay wound healing in human [18] and animal models [14,19,20,25]. The mechanisms by which steroids disturb wound healing are as follows: inhibition of angiogenesis and increase of collagen fiber, and suppression of mRNA expression of keratinocyte growth factor, which play an important role in the morphogenesis of epithelium and wound re-epithelialization, at the wound site [14,19,20,25]. Furthermore, when bacterial or viral infection is observed, steroids cannot be used because they may make infectious diseases worse. These effects of steroids are disadvantageous in the treatment of allergic dermatitis.

Itching and scratching are important factors in the development of allergic dermatitis. Spontaneous development of dermatitis is preceded by scratching in NC/Nga mice [27], and cutting off of the toenails in NC/Nga mice has a therapeutic effect and inhibits the development of dermatitis [28]. This suggests that physical stress on the skin that is associated with scratching is a significant factor in the development of dermatitis in NC/Nga mice. In these mice, pretreatment with dexamethasone, which is a corticosteroid, significantly suppresses scratching in NC/Nga mice [29]. The corticosteroid is effective at reducing itching as a result of inflammatory changes of the skin. Furthermore, in allergic dermatitis accompanied by itching, micro scratches invisible to the eyes are assumed to occur at the beginning of treatment. In such cases, steroids can suppress inflammation and stop itching and scratching behavior, but delay cure for micro wounds. Delayed wound healing would be one of the exacerbation factors of steroid-induced skin atrophy. Therefore, drugs with anti-inflammatory, pruritus suppressing, and wound healing effects are preferred.

The roots of *Alkanna tinctoria* (AT) and LE have been used for the treatment of many diseases since ancient times because of their various biological activities. In the 4th–5th century BC, Hippocrates, a Greek doctor and philosopher, used AT for the treatment of skin ulcers [1,3]. In the 2nd century AD, Hua Tuo, a Chinese surgical pioneer and herbal expert, used LE as a traditional Chinese therapy [1,3]. These therapeutic properties are due to naphthoquinone derivatives, such as alkaline extracted from AT and shikonin, and optical isomer of alkaline extracted from LE [1,3].

In this study, we focused on the beneficial effects of shikonin in the treatment of allergic dermatitis such as AD. Shikonin has anti-inflammatory activity on both acute and chronic inflammatory diseases in vitro [4,5] and in vivo [6]. Using a mouse model of allergic dermatitis, consistent with a previous study, we showed that shikonin significantly improved the clinical severity of allergic dermatitis by inhibiting inflammation. Furthermore, it reduced the frequency of scratching. Similarly, shikonin significantly prevented the clinical severity of allergic dermatitis. Several pharmacological mechanisms of shikonin against inflammation have been reported as follows: shikonin inhibits histamine release from human basophils through inhibition of Syk-dependent phosphorylation [7]; it inhibits NO production via inhibition of NO synthase induced by lipopolysaccharide in murine RAW 264.7 macrophages [8], and it inhibits the mitogen-induced mRNA and protein expression of IL-4 and IL-5 in murine EL-4 T lymphocytes [30]. Furthermore, on DNA microarray analysis shikonin inhibited the expression of inflammatory molecules, notably cytokines (TNF-α, IL-1β and IL-4), chemokines (macrophage inflammatory protein (MIP)-1β and monocyte chemotactic protein (MCP)-2), and inflammatory modulators (nuclear factor of activated T-cells, cytoplasmic 3 (NFATC3) and prostaglandin-endoperoxide synthase 2 (PTGS2)) in lipopolysaccharide-stimulated human monocyctic THP-1 cells [31]. Of these, IL-4 is a key Th-helper (Th) type 2 cytokine critical for Th2 cell differentiation, IgE production and eosinophil recruitment, and plays pivotal roles in the development of AD [32]. These reports suggest that shikonin improves the clinical severity of allergic dermatitis through inhibiting the expression of these inflammatory molecules around inflammatory sites.

Shikonin promoted wound healing in comparison with Vaseline, and this was associated with reduced IL-1β expression and elevated bFGF expression. Given that bFGF is known to promote fibroblast proliferation, the increased expression of bFGF by shikonin may be partially responsible for the enhancement of wound healing. Another previous study in humans showed that the root of LE extract significantly improved skin barrier function by decreasing transepidermal water loss in a time- and dose-dependent manner [33], indicating the potential effects of LE, probably shikonin. Taken together, the present results strongly support the use of shikonin as a potential beneficial alternative to steroids for treatment of allergic skin diseases including AD.

In conclusion, shikonin reduces scratching, improves skin lesions, and prevents the onset of allergic dermatitis. It also promoted wound healing in a mouse model of skin wound. We believe that the present study makes a significant contribution to the literature because it demonstrates that shikonin
is a potential beneficial alternative to steroids for the treatment of allergic skin diseases including AD.

ACKNOWLEDGMENTS

We thank S. Yanagisawa for statistical analysis. This research was supported, in part, by a grant from The New Industry Research Organization (NIRO), Japan (to T.T.).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Papageorgiou VP, Assimopoulou AN, Couladouros EA, Hepworth D, Nicolaou KC. The chemistry and biology of alkannin, shikonin, and related naphthazarin natural products. Angew. Chem. Int. Ed. Engl. 1999; 38: 270–301.
2. Chen X, Yang L, Oppenheim JJ, Howard MZ. Cellular pharmacology studies of shikonin derivatives. Phytother. Res. 2002; 16: 199–209.
3. Papageorgiou VP, Assimopoulou AN, Ballis AC. Alkannins and shikonins: A new class of wound healing agents. Curr. Med. Chem. 2008; 15: 3248–3267.
4. Kourounakis AP, Assimopoulou AN, Papageorgiou VP, Gavalas A, Kourounakis PN. Alkannin and shikonin: Effect on free radical processes and on inflammation – a preliminary pharmacological investigation. Arch. Pharm. (Weinheim) 2002; 335: 262–266.
5. Chiu SC, Yang NS. Inhibition of tumor necrosis factor-α by human basophils and Syk kinase activity. J. Biol. Chem. 2004; 279: 5877–5885.
6. Staniforth V, Wang SY, Shyur LF, Yang NS. Shikonins, phytochemicals from Lithospermum erythrorhizon, inhibit the transcriptional activation of human tumor necrosis factor alpha promoter in vivo. J. Biol. Chem. 2007; 282: 1640–1645.
7. Takano-Ohmuro H, Yoshida LS, Yuda Y, Morioka K, Kitani S. Shikonin inhibits IgE-mediated histamine release by human basophils and Syk kinase activity. Inflamm. Res. 2008; 57: 484–488.
8. Yoshida LS, Kawada T, Irie K, Yuda Y, Hirai T, Ikemoto F, Takano-Ohmuro H. Shikonin directly inhibits nitric oxide synthase: Possible targets that affect thoracic aorta relaxation response and nitric oxide release from RAW 264.7 macrophages. J. Pharmacol. Sci. 2010; 112: 343–351.
9. Yoshida LS, Kakegawa T, Yuda Y, Takano-Ohmuro H. Shikonin changes the lipopolysaccharide-induced expression of inflammation-related genes in macrophages. J. Nat. Med. 2017; 71: 723–734.
10. Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. J. Clin. Invest. 2004; 113: 651–657.
11. Williams HC. Clinical practice. Atopic dermatitis. N. Engl. J. Med. 2005; 352: 2314–2324.
12. Li C, Lasse S, Lee P et al. Development of atopic dermatitis-like skin disease from the chronic loss of epidermal caspase-8. Proc. Natl. Acad. Sci. U. S. A. 2010; 107: 22249–22254.
13. Akers WA. Risks of unoccluded topical steroids in clinical trials. Arch. Dermatol. 1980; 116: 786–788.
14. Anstead GM. Steroids, retinoids, and wound healing. Adv. Wound Care 1998; 11: 277–285.
15. Suto H, Matsuda H, Mitsuaki K et al. NC/Nga mouse: A mouse model for atopic dermatitis. Int. Arch. Allergy Immunol. 1999; 120: 70–75.
16. Tsudzuki M, Watanabe N, Wada A, Nakane Y, Hiroi J, Matsuda H. Genetic analyses for dermatitis and IgE hyperproduction in the NC/Nga mouse. Immunogenetics 1997; 47: 88–90.
17. Yamamoto M, Haruna T, Ueda C et al. Contribution of itch-associated scratch behavior to the development of skin lesions in Dermatophagoides farinae-induced dermatitis model in NC/Nga mice. Arch. Dermatol. Res. 2009; 301: 739–746.
18. Ragan C, Grokoest AW, Boots RH. Effect of adrenocorticotropic hormone on rheumatoid arthritis. Am. J. Med. 1949; 7: 741–750.
19. Brauchle M, Fassler R, Werner S. Suppression of keratinocyte growth factor expression by glucocorticoids in vitro and during wound healing. J. Invest. Dermatol. 1995; 105: 579–584.
20. Wang AS, Armstrong EJ, Armstrong AW. Corticosteroids and wound healing: Clinical considerations in the perioperative period. Am. J. Surg. 2013; 206: 410–417.
21. Karayannopoulou M, Tsili V, Loukopoulos P et al. Evaluation of the effectiveness of an ointment based on alkannins/shikonins on second intention wound healing in the dog. Can. J. Vet. Res. 2011; 75: 42–48.
22. Yin SY, Peng AP, Huang LT, Wang YT, Lan CW, Yang NS. The phytochemical shikonin stimulates epithelial-mesenchymal transition (EMT) in skin wound healing. Evid. Based Complement. Alternat. Med. 2013; 2013: 262796.
23. Yan Y, Furumura M, Gouya T et al. Shikonin promotes skin cell proliferation and inhibits nuclear factor-kappaB translocation via proteasome inhibition in vitro. Chin. Med. J. (Engl) 2015; 128: 2228–2233.
24. Andujar I, Rios JL, Giner RM, Recio MC. Shikonin promotes intestinal wound healing in vitro via induction of TGF-beta release in IEC-18 cells. Eur. J. Pharm. Sci. 2013; 49: 637–641.
25. Schacke H, Docke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol. Ther.* 2002; **96**: 23–43.

26. Schoepe S, Schacke H, May E, Asadullah K. Glucocorticoid therapy-induced skin atrophy. *Exp. Dermatol.* 2006; **15**: 406–420.

27. Takahashi N, Arai I, Honma Y et al. Scratching behavior in spontaneous- or allergic contact-induced dermatitis in NC/Nga mice. *Exp. Dermatol.* 2005; **14**: 830–837.

28. Hashimoto Y, Arai I, Nakanishi Y, Sakurai T, Nakamura A, Nakaike S. Scratching of their skin by NC/Nga mice leads to development of dermatitis. *Life Sci.* 2004; **76**: 783–794.

29. Takano N, Arai I, Hashimoto Y, Kurachi M. Evaluation of antipruritic effects of several agents on scratching behavior by NC/Nga mice. *Eur. J. Pharmacol.* 2004; **495**: 159–165.

30. Lee CC, Kang JJ, Chiang BL, Wang CN, Cheng YW. Shikonin inhibited mitogen-activated IL-4 and IL-5 production on EL-4 cells through downregulation of GATA-3 and c-Maf induction. *Life Sci.* 2011; **89**: 364–370.

31. Chiu NS, Tsao SW, Hwang PI, Vanisree S, Chen YA, Yang NS. Differential functional genomic effects of anti-inflammatory phytocompounds on immune signaling. *BMC Genomics* 2010; **11**: 513.

32. David Boothe W, Tarbox JA, Tarbox MB. Atopic dermatitis: Pathophysiology. *Adv. Exp. Med. Biol.* 2017; **1027**: 21–37.

33. Chang MJ, Huang HC, Chang HC, Chang TM. Cosmetic formulations containing *Lithospermum erythrorhizon* root extract show moisturizing effects on human skin. *Arch. Dermatol. Res.* 2008; **300**: 317–323.