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Raepenol™ Cream, a Complex of Natural Compounds, Promotes Wound Healing and Relieves Pruritus *In Vivo*

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Abstract. *Background/Aim: Skin wound healing is a physiological process restoring the structural and functional integrity of injured skin. During this process, wound management preventing bacterial infection and complications is important for the regeneration of skin layers and adnexa, as well as the protective function of the skin. Therefore, the development of an effective ointment to promote wound healing without complications is beneficial. Materials and Methods: This study developed Raepenol™ cream, comprising a base cream and natural compounds including paeonol, D-panthenol and extract of Centella asiatica, and assessed its therapeutic effect in wound healing. A rat model of skin wound healing and a mouse model of imiquimod-induced pruritus were employed. The effect of Raepenol™ cream was evaluated by wound size and histological analysis, including the integrity of skin structures and inflammatory response. Results: Raepenol™ cream treatment effectively restored the structural integrity of the skin in rats, including wound closure, regeneration of skin adnexa, and reconstitution of collagen, comparable to commercial ointment. Additionally, Raepenol™ cream significantly suppressed pruritus by inhibiting mast cell infiltration or retention in the inflammatory site of mouse ears. Conclusion: Raepenol™ cream effectively promoted wound healing and relieved pruritus in animal models. These results suggest that it could be a promising option for wound care and pruritus relief, offering potential advantages over current ointments.*

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Skin wound healing is a complex process aimed at repairing damaged tissue and restoring skin integrity (1). Wound healing is achieved through sequential and overlapping processes, including hemostasis, inflammation, proliferation and remodeling (2-4). Hemostasis is an immediate response to tissue damage initiated by the release of platelets from the site of the microvascular injury and their interaction with the basement membrane and extracellular matrix (ECM). These interactions trigger platelet activation and the coagulation cascade, causing contraction of damaged blood vessels and formation of blood clots to prevent bleeding from the wound

Table I. *Composition of the vehicle cream and Raepenol™ cream.*

Ointment	Components
Vehicle	Cetyl alcohol (2%), liquid paraffin (15%), glycerin (1%), propylene glycol (8%), sorbitan stearate (3%), polysorbate-60 (4%) and distilled water.
Raepenol™	Paeonol (1%), D-panthenol (2%), *Centella asiatica* extract (1%), and herbal plant complex (0.05%) consisting of lyophilized powder of *Echinacea purpurea* flower, *Rhus semialata* leaf/stem, Saururus chinensis leaf and *Artemisia princeps*.

(5). In the inflammatory phase, numerous cellular and soluble components of the immune system are recruited to damaged tissue and engaged to eliminate pathogens from the wound. Wound-induced inflammation persists only for a few days in normal wound healing. However, an excessive or inefficient immune response causes chronic inflammation in the wound, which is associated with complications, such as ulceration and hyperkeratosis (6-9). During proliferation, various orchestrated reactions of skin cells repair the damage and form granulation tissue through processes, such as fibroplasia, re-epithelialization, angiogenesis, peripheral nerve regeneration, and inflammation resolution. During ECM remodeling, the last phase of wound healing, immune cells and fibroblasts are removed from the tissue, and this is followed by collagen reconstitution lasting several months. This process replaces temporal granulation tissue with a permanent scar that restores the functional integrity of the skin (10-12). However, wound-healing impairment or dysregulation can cause complications and discomfort, such as infection, fibrosis, chronic wounds, and pain (8, 13).

Chronic wounds often exhibit incomplete healing and persistent inflammation, leading to pruritus (itching). Inflammatory mediators, especially histamine released from mast cells, play a crucial role in skin wound healing. However, they can cause pruritus by increasing blood flow and permeability of vessels in the wound (14-16). Pruritus should be addressed in wound care, as its persistence can lead to scratching, potentially causing secondary damage to the wound (17).

Paeonol, a natural phenolic compound isolated from *Paeonia suffruticosa* (Moutan or Tree Peony), possesses antioxidant, anti-inflammatory, antibacterial, and analgesic effects. It effectively inhibits oxidative injury and inflammatory responses and alleviated pain in a rat model of hepatocellular carcinoma and mouse model of dermal thermal hyperalgesia (18-23). *Centella asiatica*, a medicinal herb, has antioxidant and anti-inflammatory properties (24-27). Its extract promoted wound repair in a rat model of oral mucosa regeneration (28). D-Panthenol, a natural antioxidant widely used for skincare, improves pruritus and skin regeneration by enhancing epidermal differentiation (29-33).

Although the intricate process of wound healing has been extensively studied, a crucial need for innovative approaches to enhance this natural restorative mechanism remains. Therefore, we introduce a novel ointment, Raepenol™, composed of paeonol, *C. asiatica* extract, D-panthenol, and other supplements. Our study aimed to assess the efficacy of Raepenol™ cream in promoting skin wound healing and relieving pruritus through comprehensive *in vivo* experiments.

Materials and Methods

Preparation of ointments. Fucidin ointment (Donghwa Pharmaceutical Co., Seoul, Republic of Korea) and Madecassol care ointment (Dongkook Pharmaceutical Co., Seoul, Republic of Korea) were used as reference materials. A base cream used as a vehicle control in the experiments was created by mixing cetyl alcohol, liquid paraffin, glycerin, propylene glycol, sorbitan stearate, polysorbate-60, 1,2-hexanediol, and distilled water at 80˚C using a homogenizer. Raepenol™ cream was formulated by incorporating paeonol (Plamed Green Science Group, Xi'an, P.R. China), D-panthenol (Xinfa Pharmaceutical Co. Ltd, Shangdong, P.R. China), a *C. asiatica* extract (World-Way Biotech Inc., Changsha, P.R. China), and a herbal plant complex (Herbmaul Inc., Chungju, Republic of Korea) into the base cream. The herbal plant complex included in Raepenol™ cream comprised equal amounts of high-pressure, hot water-extracted lyophilized powder of *Echinacea purpurea* flower, *Rhus semialata* leaf/stem, *Saururus chinensis* leaf and *Artemisia princeps*. Table I presents the compositions of the vehicle and Raepenol™ cream.

Establishment of a rat model of skin wound healing. Twenty Sprague-Dawley rats (male, 4-5 weeks old, with a mean weight of 162±2 g) were used as the full-thickness, circular skin replacement models. The rats were purchased from Samtako Bio Korea (Osan, Republic of Korea) and acclimatized for 7 days before the experiment. The rats were maintained at 22˚C±2˚C and 50%±10% humidity on a 12-h light/dark cycle and were freely provided with tap water and commercial food. The rats were divided into four groups, each consisting of five rats per group: Base cream-(vehicle), Raepenol™ cream-, Fucidin ointment-, and Madecassol ointment-treated groups. The rats were anesthetized with 2.5% avertin (Sigma-Aldrich, St. Louis, MO, USA) and hair was removed using a hair removal cream (Body Natur, Seoul, Republic of Korea). Two full-thickness skin wounds were created parallel to the dorsal region with an 8-mm diameter biopsy punch. The ointments (0.1 g) were applied immediately after wound creation and were covered by gauze. The ointments and gauze were removed on day 9. The wounds were photographed on days 0, 3, 6, and 9 to record wound closure. The wounds were then sampled using a biopsy punch under

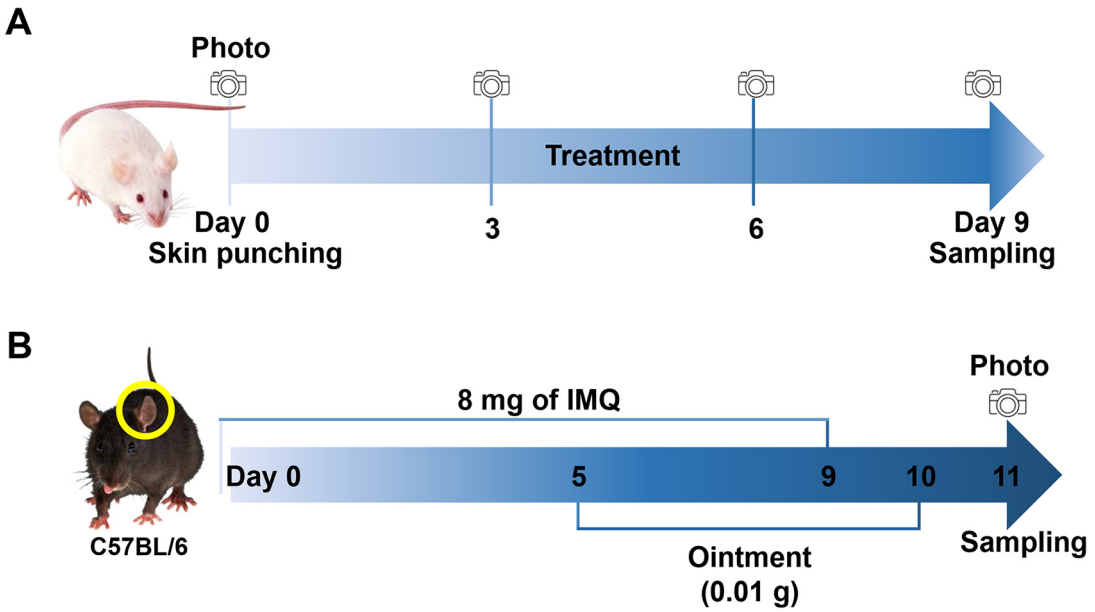

Figure 1. *The experimental procedure for the treatment of the rat wound-healing model (A) and for the imiquimod (IMQ)-induced mouse model of pruritus (B). Treatments were Vaseline (normal), Fucidin ointment, Madecassol ointment, base cream (vehicle), or Raepenol™ cream.*

avertin anesthesia. Figure 1A shows the establishment of the wound-healing rat model.

Establishment of a mouse model of pruritus. Twenty C57BL/6 mice (male, 4-5 weeks old, with a mean weight of 19±2 g) were purchased from Samtako Bio Korea and acclimatized for 7 days before the experiment. The mice were randomly divided into five groups (n=5/group): normal (Vaseline-treated group, experimental control for imiquimod-treated animals), base cream- (vehicle), Raepenol™ cream-, Fucidin ointment-, and Madecassol ointment-treated groups. The mice were maintained at 22˚C±2˚C and 50%±10% humidity in a 12-h light/dark cycle and were freely provided with tap water and commercial food. The pruritus model was established using treatment with 8 mg of imiquimod cream (5%) (Dona ST, Seoul, Republic of Korea) on the left ear once a day for 10 consecutive days. After imiquimod treatment, the groups received 0.01 g of cream/Vaseline, once a day from day 5 to day 10. The scratching behavior test was performed on day 10, and the ear was photographed on day 11. Ear tissues (8-mm diameter) were collected using a biopsy punch and weighed to determine edema. Figure 1B shows the establishment of the mouse model of pruritus.

Measurement of wound size. The wounds were photographed with a digital camera on days 0, 3, 6, and 9 to determine wound size. Wound sizes were calculated according to the following formula: wound size (mm^2) = (length of wound ÷ 2) × (short side of wound ÷ 2) × 3.14.

Histological analysis. Two pieces of skin tissue, each obtained from a separate wound on each rat, were collected on day 9 and fixed in 4% paraformaldehyde for 48 h, then embedded in paraffin, and cut into 4-μm thickness sections. The wound skin and ear tissues were stained with hematoxylin and eosin before determining the wound length and morphology of skin layers (epidermis, dermis, subcutaneous fat, and organelles). Skin wound tissues were stained with Masson's trichrome to analyze collagen deposition. Ear skin tissues were stained with toluidine blue before mast cell quantification. The stained tissues were observed under a microscope (magnification, ×100) and analyzed using Image J software (v1.53e; National Institutes of Health, Bethesda, MA, USA).

Assessment of scratching behavior. Scratching behavior was recorded by video after the topical application of each treatment on day 10. The number of ear scratches that induced itching using imiquimod cream was counted for 60 min.

Statistical analysis. Each treatment in this study was replicated three times. SPSS 25.0 (IBM, Armonk, NY, USA) was used for data processing and statistical analysis. One-way analysis of variance, followed by Duncan's test, was used to test for significant differences among all groups ($p<0.05$). The data are reported as the mean±standard error of the mean. Statistical differences between groups are denoted by different letters.

Institutional Review Board Statement. All animal experiments were conducted following approval from the Animal Ethics of Kyungpook National University (approval no. 2022-0007).

Results

Raepenol™ cream treatment promoted wound contraction in the rat wound-healing model. We employed the full-thickness circular skin replacement rat model to investigate the therapeutic effect of Raepenol™ cream on skin wound

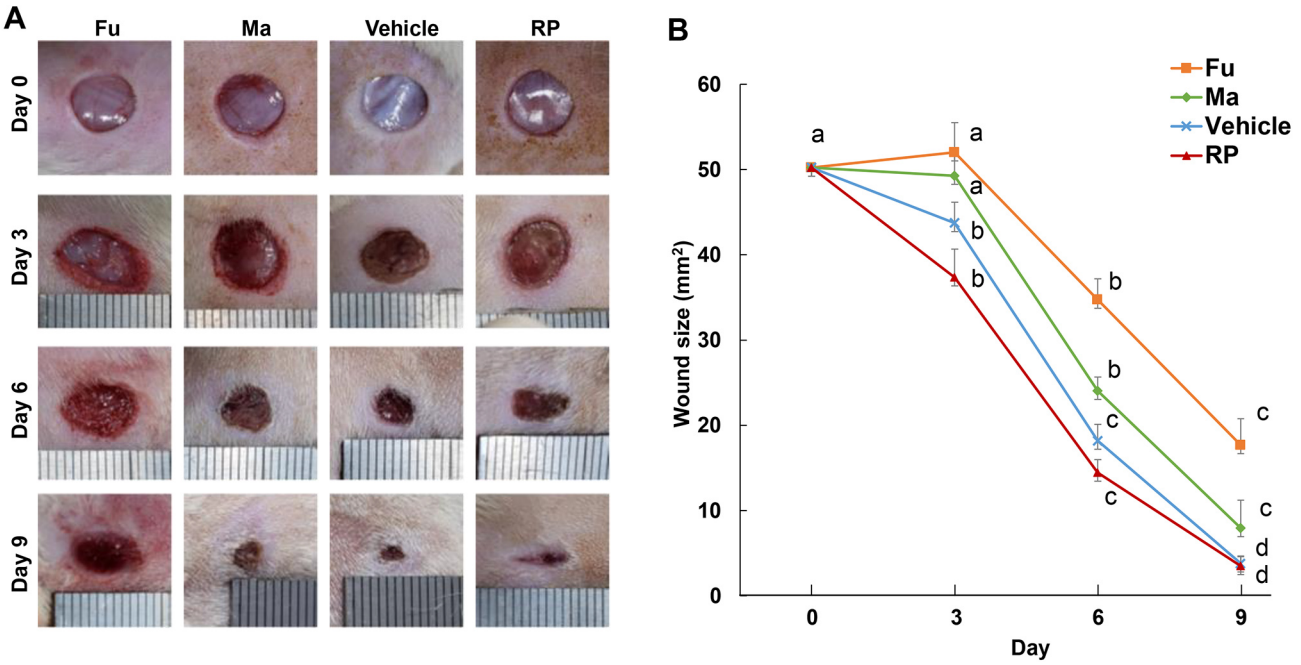

Figure 2. *Effect of Raepenol™ cream on wound contraction in the wound-healing rat model. (A) Wound images on days 0, 3, 6, and 9 after treatment with Fucidin (Fu) ointment, Madecassol ointment (Ma), base cream (vehicle), or Raepenol™ cream (RP). (B) Wound size was calculated from day 0 to 9 (n=10) using the equation: wound size (mm^2) =(length of wound ÷ 2) × (short side of wound ÷ 2) × 3.14. Data points with different letters are significantly different at $p<0.05$.*

healing. The rat model was treated with Fucidin ointment, Madecassol ointment, base cream (vehicle), or Raepenol™ cream, followed by measuring the wound size (Figure 1A). Wound contraction was accelerated in the vehicle-, and Raepenol™-treated groups compared to the groups treated with Madecassol and Fucidin (Figure 2A). Calculated wound sizes were significantly smaller in vehicle and Raepenol™ groups than in Fucidin and Madecassol groups (Figure 2B). Although there was no statistically significant difference in wound size between the vehicle- and Raepenol™-treated groups, wound contraction on days 3 and 6 was slightly better with Raepenol™ treatment.

Raepenol™ cream application improved skin regeneration. Wound skin tissues were collected on day 9 after treatment and stained with hematoxylin and eosin to analyze the impact of Raepenol™ cream treatment on skin wound healing at the histological level. The regeneration of skin layers and cellularity of the epidermis improved in the groups treated with vehicle or Raepenol™ compared to those treated with Fucidin or Madecassol (Figure 3A). Histological measurement indicated that Madecassol, vehicle, and Raepenol™ significantly reduced wound size (Figure 3B) through epidermis regeneration compared to Fucidin and (Figure 3C). In terms of dermis thickness, the Madecassol-treated group exhibited significantly lower dermis thickness compared to the other groups (Figure 3D). Subcutaneous fat, which is recovered at the end of the wound-healing process, was increased in vehicle-, and Raepenol™-treated groups (Figure 3E). Regeneration of skin adnexa, such as glands and hair follicles, needed for functional skin integrity, was significantly promoted in groups treated with Madecassol, vehicle, or Raepenol™ compared to the Fucidin-treated group (Figure 3F).

Collagen reconstitution in the dermal ECM promotes structural integrity restoration and supports the scar. Therefore, we assessed whether Raepenol™ cream treatment influences collagen synthesis and deposition using Masson's trichrome staining of the skin tissue. The group treated with vehicle exhibited significantly higher collagen synthesis and deposition in the dermis compared to those treated with Fucidin, Madecassol and Raepenol™, whilst this was true for Raepenol™ compared with Fucidin (Figure 3G and H). Moreover, collagen was well reorganized within the ECM under the epidermis in the vehicle- and Raepenol™-treated groups, indicating wound tissue remodeling and scar formation, which mark the end phase of wound healing. Thus, Raepenol™ cream showed therapeutic effects in skin wound healing. Furthermore, it was more effective than Fucidin but not superior to Madecassol or the vehicle regarding structural restoration of the wound.

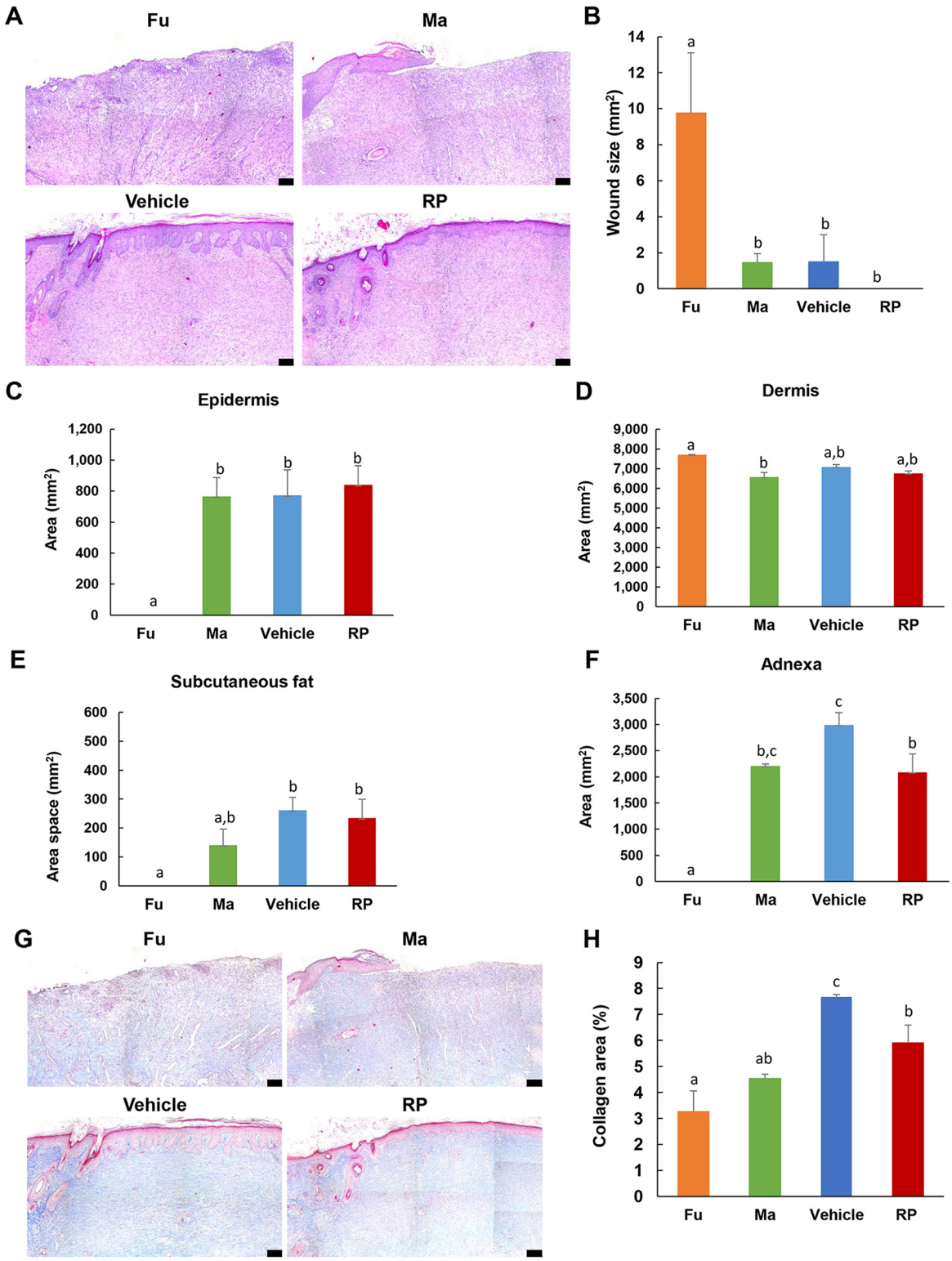

Figure 3. *The effect of treatment with Fucidin (Fu) ointment, Madecassol ointment (Ma), base cream (vehicle), or Raepenol™ cream (RP) on histological regeneration of the skin in the wound-healing rat model. (A) Images of hematoxylin and eosin-stained skin wound on day 9 (scale bar=75 μm). (B) The uncovered area of the epidermis (wound) in the tissue. (C-F) The areas of the epidermis, dermis, subcutaneous fat, and adnexa (n=3). (G) Images of Masson's trichrome-stained skin wound on day 9 (scale bar=75 μm). (H) The area of collagen was measured (n=3). Data with different letters are significantly different at p<0.05.*

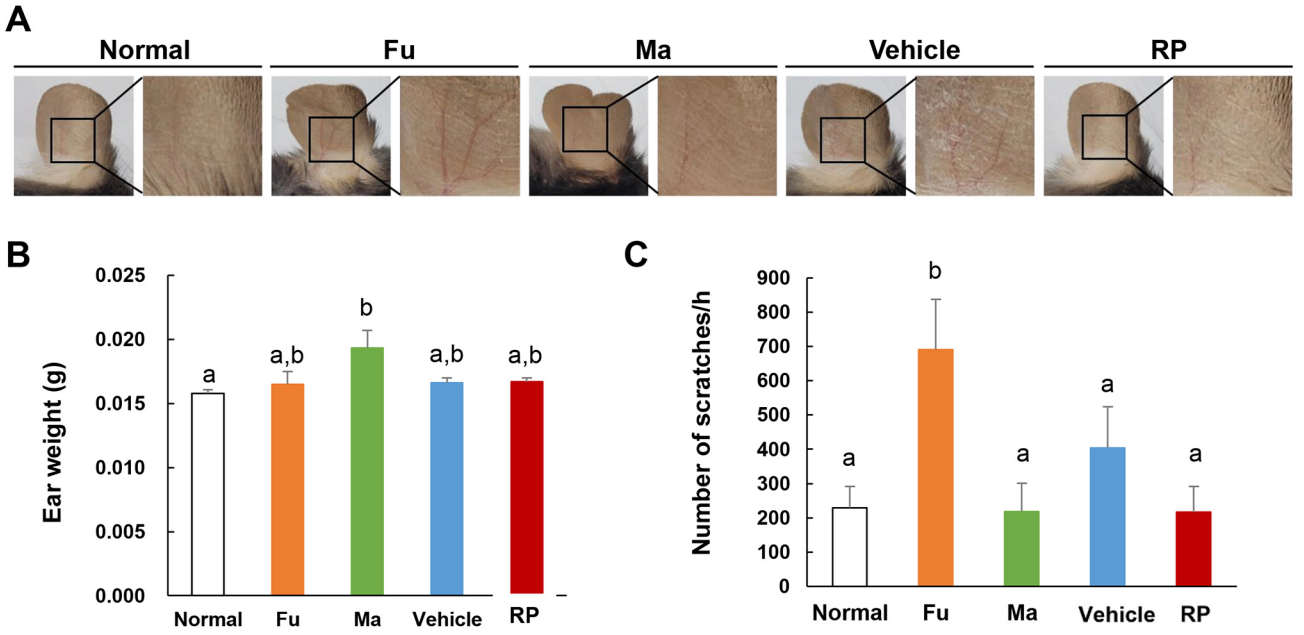

Figure 4. *Effect of treatment with Fucidin (Fu) ointment, Madecassol ointment (Ma), base cream (vehicle), or Raepenol™ cream (RP) in the imiquimod-induced mouse model of pruritus. Raepenol™ cream treatment relieved erythema and pruritus in the pruritus model. (A) Representative images of the left ears observed on day 11. (B) The weight of ear on day 11 (n ≥4). (C) The number of scratches was counted for 60 min at day 10 (n ≥4). Data with different letters are significantly different at p<0.05.*

Pruritus in mouse model was ameliorated by Raepenol™ cream treatment. We employed the imiquimod-induced pruritus mouse model to elucidate the efficacy of Raepenol™ cream in alleviating pruritus and investigated its effect following the experiment (Figure 1B). An improvement of pruritus, erythema and hyperkeratosis was observed in the Raepenol™-treated group compared to those treated with Fucidin, Madecassol, or vehicle (Figure 4A). Ear weight was measured on day 11 after treatment to assess the degree of edema. Ear weights in groups treated with Fucidin, vehicle, or Raepenol™ did not significantly differ from the normal group (Figure 4B). Additionally, the number of ear scratches caused by pruritus when mice were treated with Madecassol, vehicle, or Raepenol™ was similar to the normal group, although the number of ear scratches was significantly increased in the Fucidin-treated group (Figure 4C).

Raepenol™ cream showed anti-inflammatory activity. In line with previous observations, histological analysis revealed hyperkeratosis in the Fucidin-treated group. An increase in the epidermal thickness was also observed in groups treated with Fucidin, Madecassol, or vehicle. In contrast, Raepenol™ treatment significantly reduced the epidermal thickness, comparable to that of normal ear tissue (Figure 5A and B). As expected, whole-ear thickness was not increased in the Raepenol™-treated group (Figure 5C), in contrast to all other treatments. Thus, the Raepenol™ cream demonstrated superior potency in suppressing skin inflammation.

In the skin, mast cells serve as a primary effector, responding to tissue injury by releasing inflammatory mediators. Histamine, a major mediator released from mast cells, increases vascular permeability, leading to vasodilation, and induces inflammation during wound healing. However, excessive or prolonged action of histamine can cause pruritus in chronic wounds. Therefore, we evaluated the effect of Raepenol™ cream on the infiltration and retention of mast cells around the inflammatory site. Mast cells in mouse ear tissues were stained with toluidine blue, and their number was counted. The number of mast cells in the ear tissue was lower in the Raepenol™-treated group than in other groups. Furthermore, it was similar to that of the normal ear tissue (Figure 5D and E), suggesting that the Raepenol™ cream might alleviate pruritus in wounds by restricting the infiltration or retention of mast cells in the inflammatory site.

Discussion

The skin is a barrier protecting from environmental hazards and fluid loss to maintain homeostasis in the body. Therefore, the rapid and correct restoration of skin injuries, known as wound healing, is crucial. This complex process involves numerous cellular and secretory factors aimed at regenerating

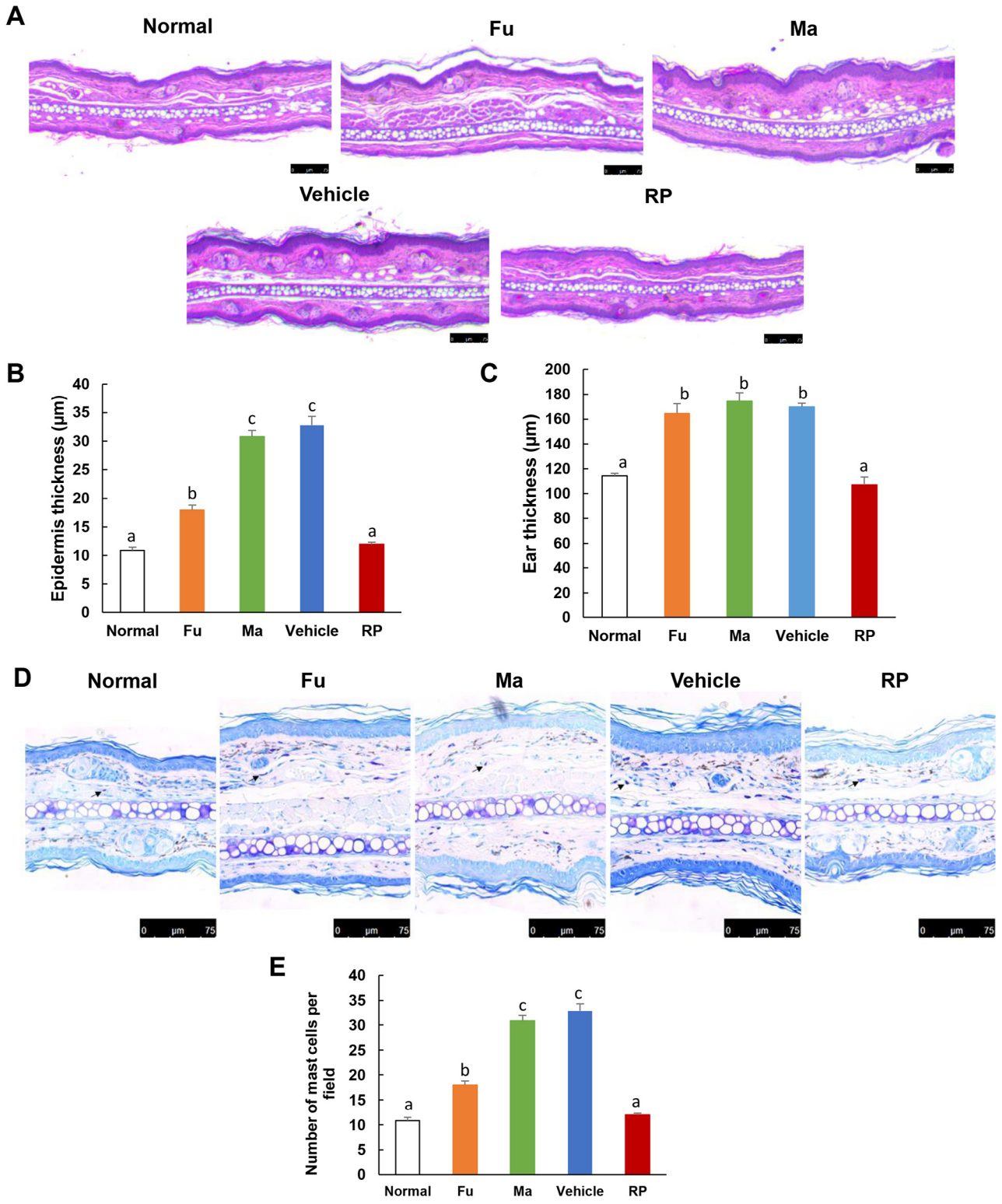

Figure 5. *Effect of treatment with Fucidin (Fu) ointment, Madecassol ointment (Ma), base cream (vehicle), or Raepenol™ cream (RP) in the imiquimod-induced mouse model of pruritus. Raepenol™ cream reduced imiquimod-induced inflammation and hyperkeratosis in the pruritus mouse model. (A) Images of hematoxylin and eosin-stained ear tissues collected on day 11 (scale bar=75 μm). (B and C) The thickness of the whole ear and epidermis on day 11 (n=3). (D) Images of ear tissues stained with toluidine blue (scale bar=75 μm). (E) The number of mast cells per field (n=4). Data with different letters are significantly different at p<0.05.*

the structural and functional integrity of the skin (3, 4, 8). However, abnormal wound healing can occur due to intrinsic and extrinsic factors, such as genetic defects, oxidative stress, aging, obesity, diabetes, pathogenic infection, and malnutrition, leading to complications of wound healing and disturbance of normal scar formation (34, 35).

There are various treatments to prevent abnormal wound healing, focusing on promoting skin regeneration and preventing infection, physical stress, and inflammation. Different ointments are used to manage skin wounds to prevent infection and excessive inflammation and promote tissue repair. However, reported issues include antibiotic resistance, pruritus, allergic reactions, and immune suppression. Consequently, there is a pressing need for the development of ointments addressing these side effects and reducing antibiotic resistance (36-39). Natural products have emerged as potential sources of wound-healing agents that can improve skin regeneration with reduced side-effects (40, 41). In line with this, combining natural compounds with commercial ointments might be beneficial to address their weaknesses.

This study evaluated the efficacy of newly developed Raepenol™ cream, containing paeonol, D-panthenol and *C. asiatica* extract, in wound healing and pruritus alleviation in experimental animal models. Raepenol™ effectively promoted wound closure and scar formation with histopathological regeneration of the skin and adnexa in the wound-healing rat model. Additionally, Raepenol™ increased collagen synthesis and deposition in the remodeling phase. In particular, Raepenol™ cream effectively relieved pruritus through its anti-inflammatory effects, inhibiting mast cell infiltration or retention and hyperkeratosis in the imiquimod-induced pruritus mouse model. These findings suggest that Raepenol™ cream might be an effective ointment with efficacy superior to commercial ointments for simultaneously treating both skin wounds and pruritus.

In this study, Raepenol™ cream promoted skin wound healing, although its efficacy was similar to Madecassol and the vehicle. Both Raepenol™ and Madecassol creams contain *C. asiatica* extract. *C. asiatica* is a medicinal plant known for its anti-inflammatory and pro-angiogenic effects during skin wound healing (42). Surprisingly, the base cream used for the vehicle control showed comparable effects with Raepenol™ cream in promoting wound contraction and skin regeneration but did not alleviate inflammation and pruritus. This unpredicted effect might arise from the moisturizing and temporal barrier effect of components in the base cream which are commonly used in skincare products. Maintaining the wound in a moist condition alone promotes wound healing (43). Although this study yielded promising results on wound healing, it is essential to acknowledge its limitations. Considering paeonol, a key component of Raepenol™ cream, is known for its antibacterial properties, we hypothesize that Raepenol™ cream might help prevent wound infection. Notably, no instances of infection were observed during the experiments. However, this assumption lacks sufficient evidence because no infection was observed in those treated with the vehicle alone. Therefore, further research with a specific focus on the antibacterial efficacy of Raepenol™ cream is warranted to validate this assumption.

Imiquimod is commonly used to establish a pruritus model in animal studies including mice (44, 45). Therefore, we used the imiquimod-induced pruritus mouse model to explore the anti-inflammatory effect of Raepenol™ cream, which reduced pruritus and hyperkeratosis. Mast cells play a crucial role in pruritus development, releasing pruritus-inducing mediators, such as histamine, serotonin, proteases and cytokines (16). Pruritus occurring during wound healing not only worsens the quality of the patient's life but can also induce secondary wounds by provoking scratching. Interestingly, Raepenol™ cream exhibited a more pronounced ability to alleviate pruritus by inhibiting mast cell infiltration or retention and abnormal differentiation of the epidermis than the currently used commercial ointments. Unlike other ointments, Raepenol™ cream contains paeonol, D-panthenol, and a herbal plant complex as therapeutic substances. D-Panthenol, contained in the Raepenol™ cream as a natural product, relieves the symptoms of skin irritation, such as skin dryness, roughness, scaling, pruritus, and erythema (29). Paeonol inhibits mast cell degranulation by suppressing immunoglobulin E in the blood. Therefore, Raepenol™ cream is potentially suitable for treating patients with severe pruritus, such as those with burns and psoriasis, as well as common wounds.

Conclusion

Raepenol™ cream promoted wound contraction with the regeneration of the skin and adnexa. Moreover, Raepenol™ cream effectively mitigated mast cell infiltration and abnormal epidermal differentiation through its potent anti-inflammatory effects, relieving pruritus and hyperkeratosis. Therefore, Raepenol™ cream might serve as a promising therapeutic intervention, not only for wound healing but also for addressing various skin disorders, including burns and psoriasis.

Conflicts of Interest

As the developer of a commercially available wound-healing product, we have the following disclosures to make in relation to the preparation of this article. Financial interest: This study assessed the effectiveness of a product called Raepenol™. H-G K and M-O K are the inventors of Raepenol™ cream and were granted a patent for it through this research project. H-G K is employed by GentriBio, Inc., the company that supports and commercializes Raepenol™. All experiments and results were conducted with scientific objectivity and integrity, free from manipulation or bias. There are no conflicts of interest among the other Authors.

Authors' Contributions

Conceptualization: N-EC, H-GK, JY, S-GL and M-OK; methodology: JY, YS, Soyoung Jang, Soyeon Jang and Z-YR; validation: EK, Soyeon Jang, HK, and KH; formal analysis: EK HK, LM and ZL; investigation: EK, N-EC, HK, LM, KH, ZL and C-YK; resources: H-GK, YS, S-KC and Z-YR; data curation; KP; writing-original draft; EK and N-EC; writing-review and editing: SP, SJ (Soyoung Jang), Z-YR, S-KC, S-GL, and M-OK; visualization: SP, C-YK and KP; supervision: S-GL and M-OK; project administration: EK, S-KC, Z-YR and M-OK; funding acquisition: S-KC and M-OK; All Authors have read and agreed to the published version of the article.

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