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백호탕추출물의 아토피피부염 유발 백서에서의 피부상피 미세환경 손상 회복

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Abstract

Recovery of Skin Epithelial Microenvironment Damage by Baekho-tang Extract in Atopic Dermatitis-Induced Mice

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Objectives

This study aimed to identify the recovery effect of Baekho-tang extract on skin epithelial microenvironment damage in atopic dermatitis-induced mice.

Methods

In this study, we used 4-week-old NC/Nga mice divided into four groups: lipid barrier elimination group (LBEG), dexamethasone-treated group after lipid barrier elimination (DEXG), Baekho-tang extract treatment group after lipid barrier elimination (BHTG) and control group (Ctrl). Each group was assigned 10 animals. We identified ceramide kinase, casepase 14, sodium hydrogen exchanger, cathelicidine, claudiin, and toll-like receptor 2 through our immunohistochemistry.

Results

We discovered that when compared to DEXG, ceramide kinase and caspase 14, which are markers of skin moisturizing factors were increased in BHTG. In the antimicrobial barrier-related markers sodium hydrogen exchanger and cathelicidine, as well as the tight junction markers claudin and toll-like receptor 2, the BHTG showed a significant increase compared to the other groups.

Conclusions

These results suggest that the Baekho-tang extract has a positive effect on strengthening the skin microenvironment by increasing the expression of several indicators of skin moisturizing factors, antimicrobial barriers, and tight junction function.

Key words: Animal study, Atopic dermatitis, Baekho-tang, microenvironment

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Ⅰ. Introduction

Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases in children, with a prevalence rate of 5.89% in Korea as of 2022[1]. AD is clinically characterized by symptoms such as pruritus and the distinctive distribution of rashes[1]. Although its exact cause has not yet been clearly identified, AD is considered a multifactorial disease caused by a combination of increased exposure to pollutants, dietary habits, and genetic factors[2]. Alterations in the microenvironment of the skin epithelium are also known to play an important role in both the development and progression of the disease[3].

The current treatment methods, such as steroid medication, are difficult to administer long-term due to potential side effects[4]. The chronic nature of AD which are characterized by repeated cycles of improvement and exacerbation, also makes treatment challenging[4]. Particularly in pediatric patients, AD can lead to psychological issues such as depression, anxiety, and attention deficit. Moreover, it can negatively impact the growth and development of children[5].

The epithelium is a complex tissue composed of various cell layers that form the outermost layer (epidermis) of the skin[6]. The epidermis, including the stratum corneum, consists of multiple layers that fortify the physical function of the skin barrier by producing moisturizing factors and maintaining tight junctions[6]. It also plays a broad role in skin regeneration and immune response regulation. In AD patients, these functions are impaired compared to normal skin, resulting in increased water loss and a more permeable environment that allows easier penetration of external pathogens[7]. Damage to the skin barrier leads to abnormal immune responses, which increase the secretion of pro-inflammatory cytokines and chemokines, perpetuating chronic inflammation and itching[8]. Therefore, restoring the microenvironment of the skin epithelium, including moisture factors and the integrity of the stratum corneum, is considered an important goal in AD treatment, and various therapeutic strategies are being studied[9].

In Korean Medicine (KM), AD is referred to as infantile eczema under terms such as “Taeseon” (胎癬) or “Naeseon” (奶癬). It is believed to arise from a combination of factors, including residual heat from birth (胎熱), invasion of external wind-heat (風熱), internal damp-heat (濕熱), congenital heat-toxicity (熱毒), weakened digestive function leading to dampness accumulation (脾虛濕盛), and insufficient bodily fluids (陰虛). Treatment in KM focuses on eliminating damp-heat (淸利濕熱), clearing heat and detoxifying (淸熱解毒), nourishing the blood and alleviating dryness (滋陰養血), with the goal of reducing itching and dermatitis while providing appropriate moisture to the dry skin[10].

This study hypothesizes that Baekho-tang may have an anti-inflammatory effect by restoring the damaged microenvironment of the skin epithelium. Baekho-tang (白虎湯) is a prescription composed of ‘*Gypsum fibrosum* (石膏)’. ‘*Anemarrhena asphodeloides* Bunge (知母)’, ‘*Oryza sativa L.* (粳米)’, and ‘*Glycyrrhiza uralensis* Fisch (甘草)’ and is known in Traditional Korean Medicine (TKM) for its effects in clearing heat and generating fluids (淸熱生津)[10]. The clearing heat effect (淸熱) is associated with alleviating inflammatory lesions, while the fluid-generating effect (生津) is related to relieving skin dryness[11]. Therefore, it is believed that the traditional medicinal effects of Baekho-tang may be related to skin moisturizing factors, such as ceramide, caspase, and claudin, as well as other factors that contribute to the restoration of the skin epithelium’s microenvironment. Previous studies by the authors demonstrated Baekho-tang’s effects in restoring the lipid barrier and reducing inflammation response[12,13]. This study aims to verify whether Baekho-tang also restores damage to the microenvironment of the skin epithelium, and meaningful results have been confirmed, which are reported in this paper.

Ⅱ. Materials and Methods

1. Materials

1) Animal

Four-week-old male NC/Nga mice, provided by JA-Bio (Seoul, Korea), were used in the experiment. To minimize

subjective bias during outcome evaluation, the mice were acclimated for two weeks and then selected for the experiment based on a body weight of 16 ± 1 g. The mice were divided into four groups: control group (Ctrl), lipid barrier elimination group (LBEG), dexamethasone administration group after lipid barrier elimination (DEXG), and Baekho-tang extract treatment group after lipid barrier elimination (BHTG), with 10 mice assigned to each group.

The animal experiment was conducted with the approval of the Gachon University Animal Ethics Committee (IACUC No. GU1-2021-IA0035-M2), and the management and use of laboratory animals followed the NIH guidelines. During the experiment, the mice's behavior was monitored, and individuals exhibiting aggressive hyperactivity or avoidance behavior were excluded from the study. All groups of mice were housed at 23 - 25 °C with 55 ± 10% humidity and a 12-hour light-dark cycle. They were freely provided with a diet of SAFE - 40 + RMM (SAFE, France) and filtered tap water without restriction.

2. Preparation of Baekho-tang

Two doses of Baekho-tang were prepared, consisting of 40 g of *Gypsum fibrosum*, 16 g of *Anemarrhena asphodeloides Bunge*, and 6 g of *Glycyrrhiza uralensis*, totaling 62 g. This was added to 1000 mL of distilled water and decocted for 3 hours, after which it was filtered. The filtrate was then concentrated to 50 mL using a rotary evaporator under reduced pressure and subsequently freeze-dried, yielding 4.03 g of extract (with a yield of 6.5%).

2. Methods

1) Lipid barrier removal and drug administration

The dorsal skin of the mice was shaved using a depilatory cream (Body Natur, Spain) and a razor. Afterward, the stratum corneum was removed by applying tape (3M, USA) to induce desquamation. To remove the lipid lamella of the stratum corneum, 500 μL of 10% sodium dodecyl sulfate (SDS; Sigma, USA) was applied, and the area was rubbed 20 times with a cotton swab. Following this, Baekho-tang extract was diluted in saline and administered orally to the BHTG group at a dose of 68 mg/kg, 0.2 mL per day, for 3 days. For comparison, dexamethasone (Sigma-Aldrich), the control drug, was also administered orally to the DEXG group at the same dose of 68 mg/kg, 0.2 mL per day, for 3 days.

2) Skin fixation and histological preparation

The skin was perfusion-fixed via the heart using vascular rinse and 10% neutral buffered formalin. The excised dorsal skin was fixed in 10% neutral buffered formalin at room temperature for 24 hours, then embedded in paraffin using standard procedures. Serial sections of 5 μm thickness were prepared. To examine the general structure of the atopic-induced skin epithelium following drug administration, hematoxylin and eosin staining was performed.

3) Immunohistochemistry

Immunohistochemical staining was performed to investigate the changes in ceramide kinase K and caspase 14, which are involved in the production of natural moisturizing factors (NMFs); sodium hydrogen antiporter 1 (sodium/hydrogen exchanger 1, NHE1), which helps maintain the skin's acidity; the antimicrobial peptide cathelicidin; the tight junction protein claudin; and toll-like receptor (TLR)-2, which affects tight junctions. Antibodies against ceramide kinase K, caspase 14, NHE1, cathelicidin, claudin, and TLR-2 were used in the staining process.

First, the skin sections underwent proteolysis for 5 minutes using proteinase K (20 μg/μL; Dako, Santa Clara, CA, USA). This was followed by blocking for 1 hour in 10% normal goat serum (Vector Lab, Burlingame, CA, USA) containing 1% fetal bovine serum (Sigma-Aldrich). The sections were then incubated with the primary antibodies—mouse anti-ceramide kinase (1:100, Santa Cruz Biotechnology Inc.), mouse anti-caspase 14 (1:100, Santa Cruz Biotechnology Inc.), mouse anti-NHE1 (1:100, Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA), mouse anti-cathelicidine (1:100, Abcam, Waltham, MA, USA), mouse anti-claudin (1:100, Santa Cruz Biotechnology Inc.), and mouse anti-TLR-2 (1:100, Abcam, Waltham, MA, USA)—in a humidified chamber at 4 °C for 72 hours.

Next, the sections were linked to the secondary antibody, biotinylated goat anti-mouse IgG (1:50, Santa Cruz Biotechnology Inc.), at room temperature for 24 hours, followed by incubation with an avidin-biotin complex kit (Vector Lab) at room temperature for 1 hour. The sections were developed using 0.05% 3,3'-diaminobenzidine and 0.01% HCl in 0.05 M tris-HCl buffer solution (pH 7.4), then counterstained with hematoxylin.

4) Image Analysis

The results of the immunohistochemical staining were quantified through image analysis using Image Pro 10 (Media Cybernetics, Rockville, MD, USA). Ten skin samples from each group were randomly selected, and images were taken at 100 x magnification. The analysis was performed by calculating the number of positive pixels (intensity 80 - 100) per 20,000,000 pixels. The data are presented as means ± standard error.

5) Statistics

Statistical analysis was performed using SPSS software (SPSS 25, SPSS Inc., Chicago, IL, USA). One-way ANOVA was conducted to verify significance ($p < 0.05$), followed by post-hoc testing using the Tukey HSD method.

Ⅲ. Results

1. Histological observation of damaged skin epithelium

In the histological examination of damaged skin epithelium, the LBEG and DEXG groups showed significant structural changes, including hyperplasia, desquamation of the stratum corneum, expansion of intercellular spaces in the basal and spinous layers, and increased basal membrane disruption. In contrast, the BHTG group exhibited less skin damage compared to the LBEG and DEXG groups (Figure 1).

2. Restoration of NMF production

The positive reaction for ceramide kinase was observed in the granular and spinous layers. To assess the activity of ceramide kinase in the skin, the ceramide kinase-positive reaction was measured, showing an increase in LBEG (24,259 ± 493 / 20,000,000 pixels), DEXG (33,633 ± 660 / 20,000,000 pixels), and BHTG (44,424 ± 1,065 / 20,000,000 pixels) compared to the control group (12,578 ± 394 / 20,000,000 pixels). LBEG showed a 93% increase, DEXG a 167% increase, and BHTG a 253% increase compared to the control. The ceramide kinase-positive reaction in BHTG was significantly higher, showing an 83% increase compared to LBEG and a 32% increase compared to DEXG (Figure 2).

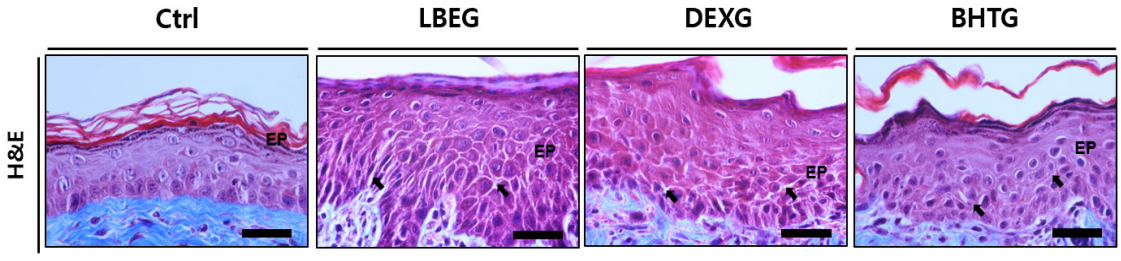

Figure 1. The mitigative effects of skin lesions by Baeckhotang (BHT) extract treatment.

The BHT extract treatment relieved symptom as enlargement of intercellualr space (arrow) in BHTG compared with LBEG and DEXG. *Abbreviations*: BHT; Baeckho-tang, Ctrl; normal, LBEG; lipid barrier eliminated group, DEXG; dexamethasone (Dx) administration group after lipid barrier elimination, BHTG; BHT extract administration group after lipid barrier elimination, H&E; hematoxylin & eosin, EP; epithelium. Arow, Intracelluar space: Bar size, 50㎛.

The positive reaction for caspase 14 was also observed in the granular and spinous layers. The caspase 14-positive reaction showed a decrease in LBEG (34,328 ± 622 / 20,000,000 pixels) compared to the control group (24,725 ± 764 / 20,000,000 pixels), while DEXG (36,953 ± 588 / 20,000,000 pixels) and BHTG (45,228 ± 569 / 20,000,000 pixels) showed an increase. LBEG showed a 39% increase, DEXG a 49% increase, and BHTG an 83% increase compared to the control group. The caspase 14-positive reaction in BHTG was significantly higher, showing a 32% increase compared to LBEG and a 23% increase compared to DEXG (Figure 2).

3. Restoring the antimicrobial barrier

The NHE-positive reaction was observed in the stratum corneum and granular layers. The NHE-positive reaction in LBEG (19,315 ± 619 / 20,000,000 pixels) decreased compared to the control group (28,516 ± 619 / 20,000,000 pixels), while it increased in DEXG (34,678 ± 798 / 20,000,000 pixels) and BHTG (48,493 ± 609 / 20,000,000 pixels). LBEG showed a 32% decrease compared to the control, while DEXG and BHTG showed 22% and 70% increases, respectively, compared to the control. The NHE-positive reaction in BHTG significantly increased by 151% compared to LBEG and by 40% compared to DEXG (Figure 3).

The cathelicidine-positive reaction was observed in the stratum corneum and granular layers. The distribution of cathelicidine in the skin showed that the cathelicidine-positive reaction decreased in LBEG (14,381 ± 462 / 20,000,000 pixels), DEXG (28,825 ± 399 / 20,000,000 pixels), and BHTG (40,875 ± 671 / 20,000,000 pixels) compared to the control (52,190 ± 485 / 20,000,000 pix-

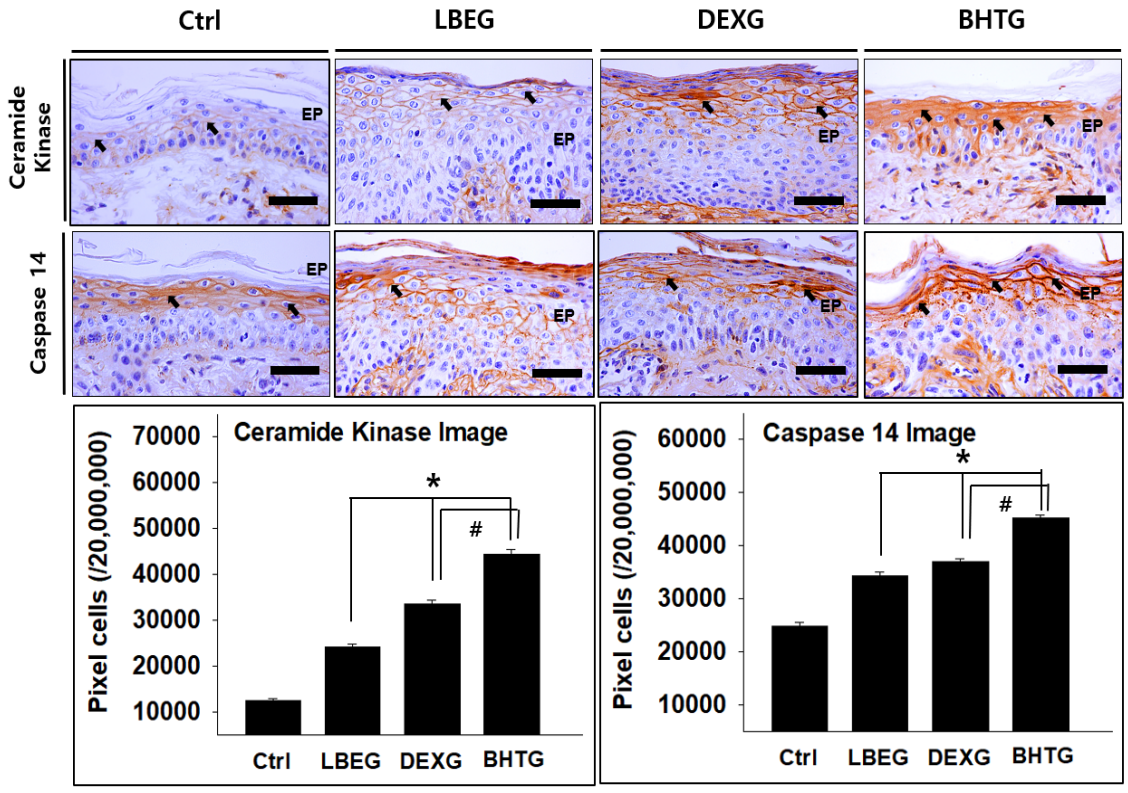

Figure 2. The regeneration of normal moisturizing factor by BHT extract treatment (Ceramide kinase and caspase 14 immunohistochemistry).

The activation of ceramide kinase and caspase 14 (arrow indicates light brown particle) was significantly increased in BHTG as compared with LBEG and DEXG, the data of ceramide kinase and caspase 14 image analysis showed the same results (*; $p < 0.05$ compared with LBEG, #; $p < 0.05$ compared with DEXG). *Abbreviations*: BHT; Baeckho-tang, Ctrl; normal, LBEG; lipid barrier eliminated group, DEXG; dexamethasone (Dx) administration group after lipid barrier elimination, BHTG; BHT extract administration group after lipid barrier elimination, H&E; hematoxylin & eosin, EP; epithelium.

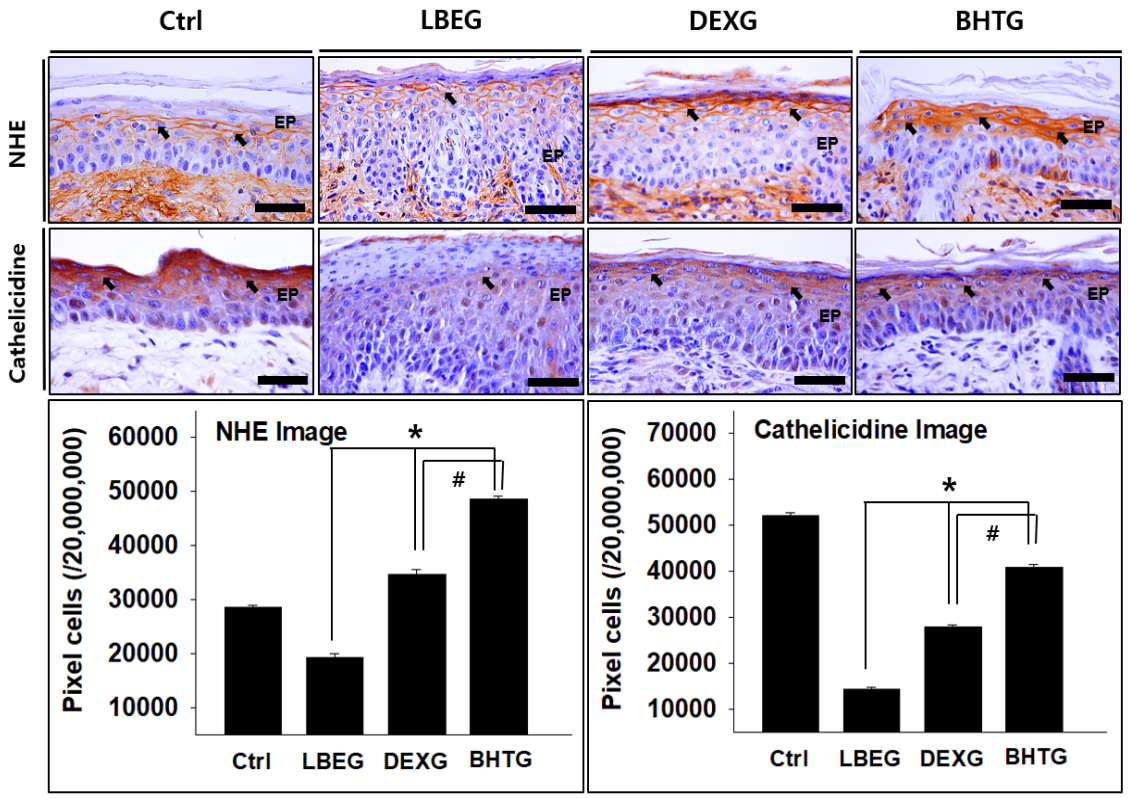

Figure 3. The regeneration of anti-microbial barrier by BHT extract treatment (NHE and cathelidine immunohistochemistry).

The activation of NHE & cathelidine (arrow indicates light brown particle) was significantly increased in BHTG as compared with LBEG and DEXG, the data of NHE and cathelidine image analysis showed the same results. (*, $p < 0.05$ compared with LBEG; #, $p < 0.05$ compared with DEXG). *Abbreviations*: BHT; Baeckho-tang, Ctrl; normal, LBEG; lipid barrier eliminated group, DEXG; dexamethasone (Dx) administration group after lipid barrier elimination, BHTG; BHT extract administration group after lipid barrier elimination, H&E; hematoxylin & eosin, EP; epithelium, NHE; sodium hydrogen exchanger.

els). LBEG showed a 72% decrease, DEXG a 47% decrease, and BHTG a 22% decrease compared to the control (Figure 3).

4. The improvement of tight junction function

After conducting immunohistochemical staining and image analysis to examine the distribution changes of claudin, the positive reaction for claudin was observed in the stratum corneum and granular layers. The distribution of claudin in the skin showed that the claudin-positive reaction decreased in LBEG (9,509 ± 293 / 20,000,000 pixels) and DEXG (18,691 ± 324 / 20,000,000 pixels) compared to the control group (25,563 ± 578 / 20,000,000 pixels), while it increased in BHTG (35,419 ± 947 / 20,000,000 pixels). LBEG showed a 63% decrease, DEXG a 27% decrease, and BHTG a 39% increase compared to the control. The claudin-positive reaction in BHTG significantly increased by 272% compared to LBEG and by 89% compared to DEXG (Figure 3).

The TLR-2 positive reaction was also observed in the granular layer. The TLR-2 positive reaction decreased in LBEG (10,503 ± 451 / 20,000,000 pixels) and DEXG (19,948 ± 734 / 20,000,000 pixels) compared to the control group (32,038 ± 434 / 20,000,000 pixels), while it increased in BHTG (47,904 ± 612 / 20,000,000 pixels). LBEG showed a 67% decrease, DEXG a 38% decrease, and BHTG a 50% increase compared to the control. The TLR-2 positive reaction in BHTG significantly increased by 356% compared to LBEG and by 140% compared to DEXG (Figure 4).

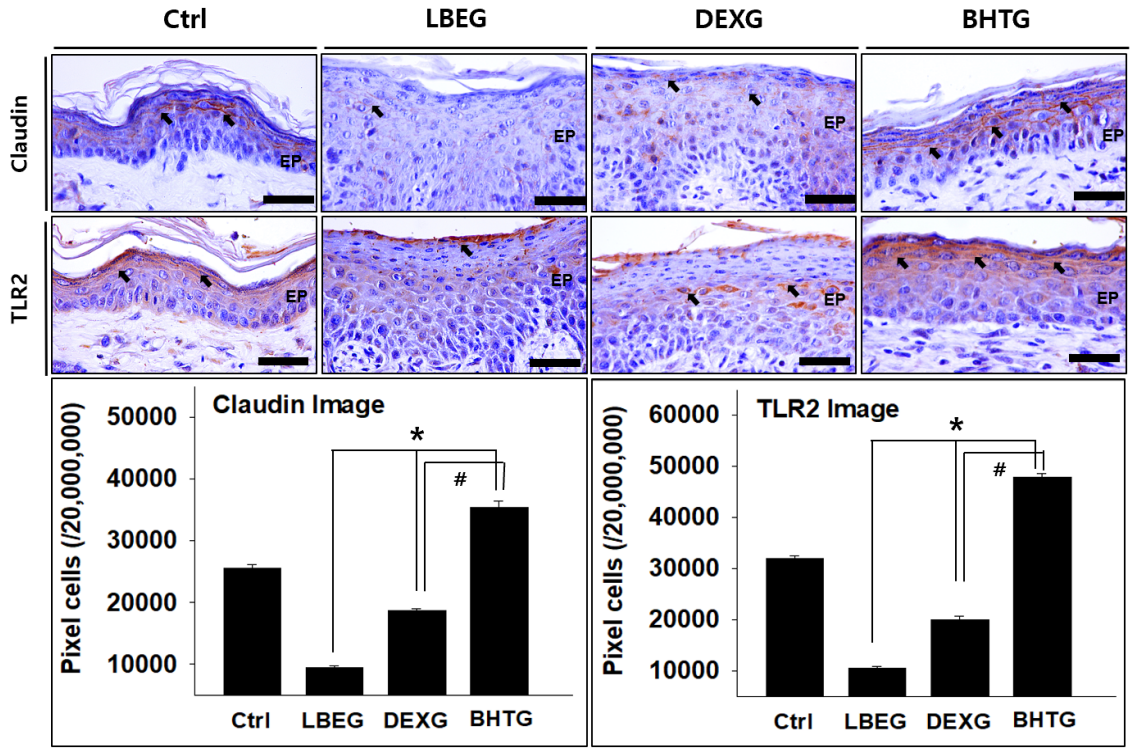

Figure 4. The regenation of tight junction by BHT extract treatment (Claudin and TLR2 immunohistochemistry).

The activation of claudin and TLR2 (arrow indicates light brown particle) was significantly increased in BHTG as compared with LBEG and DEXG, the data of claudin and TLR-2 image analysis showed the same results. (*, $p < 0.05$ compared with LBEG; #, $p < 0.05$ compared with DEXG). *Abbreviations*: BHT; Baeckho-tang, Ctrl; normal, LBEG; lipid barrier eliminated group, DEXG; dexamethasone (Dx) administration group after lipid barrier elimination, BHTG; BHT extract administration group after lipid barrier elimination, H&E; hematoxylin & eosin, EP; epithelium, TLR2; toll-like receptor 2.

Ⅳ. Discussion

Our study found that the skin moisturizing factors, antimicrobial barrier markers, and tight junction-related indicators were significantly higher in the BHTG group compared to the other groups. Particularly, BHTG showed a more pronounced increase than DEXG in all results. Additionally, in terms of the tight junction marker such as claudin and TLR-2 showed an increase in positivity in the BHTG, compared to a decrease in all other groups. We previously demonstrated that Baekho-tang is effective in regulating Th2 differentiation through the restoration of the skin lipid barrier[12]. While that study showed Baekho-tang's efficacy in skin barrier recovery with a focus on broader anti-inflammatory effects, including T helper 2 (Th2) immune response modulation[12], this study focuses on strengthening moisturizing and antimicrobial defense factors within the skin microenvironment. This study provides evidence the Baekho-tang contributes to the multidimensional recovery of skin barrier function.

The epidermis of the skin is composed of layers, including the basal, spinous, granular, and stratum corneum layers, with keratinocytes making up 95% of epidermal cells[14]. As keratinocytes move from the basal to upper layers and undergo differentiation, they form the cornified envelope, creating a strong skin barrier. Filaggrin is one of the proteins found in keratinocytes, and as the cells mature into the stratum corneum, filaggrin is converted into NMFs like pyrrocarboxylic acid and trans-urocanic acid[15]. Caspase-14, an enzyme aiding this conversion, plays a crucial role in maintaining moisture and reinforcing the skin barrier[16]. Previous studies have reported that in inflammatory skin diseases characterized by an elevated Th2 cytokine environment, caspase-14 mRNA levels significantly decrease, making caspase-14 a valuable marker for assessing epithelial conditions in such diseases[17,18].

Another important NMF in the skin is ceramide, a key lipid component that maintains the integrity of the skin barrier. In patients with AD, the amount and composition of ceramide in the stratum corneum differ from those in healthy skin. Ceramide kinase is an enzyme that converts ceramide into ceramide-1-phosphate (C1P), which strengthens the moisture retention of the skin barrier[19]. Moreover, C1P not only improves moisture but also inhibits the release of pro-inflammatory cytokines by blocking Nuclear factor kappa B (NF-κB), thus reducing inflammation[20]. In our study, we observed a significantly higher ceramide kinase response in the BHTG compared to the DEXG and ctrl, indicating that Baekho-tang may contribute to skin moisturizing and inflammation reduction by promoting ceramide kinase expression.

Another NMF identified in our study is caspase-14. This enzyme is involved in the processing of filaggrin monomers, which are broken down into hygroscopic amino acids that act as natural moisturizers[21]. Previous studies have reported that caspase-14 messenger ribonucleic acid (mRNA) levels are significantly reduced in response to Th2-related cytokines in keratinocytes, and caspase-14 expression is similarly decreased in the skin of patients with inflammatory skin diseases such as AD and psoriasis. Moreover, research shows that these reductions are consistently observed across various inflammatory skin conditions[16,22]. Thus, caspase-14 levels may provide insights into the inflammation and barrier dysfunction associated with skin diseases. Our results showed that caspase-14 levels were higher in the BHTG compared to the DEXG and ctrl. Therefore, Baekho-tang appears to enhance skin barrier function by increasing the expression of key moisturizing factors.

Our research also found that antimicrobial barrier markers were significantly higher in the BHTG compared to the DEXG and ctrl. Antimicrobial peptides naturally produced in the skin include cathelicidin, defensins, psoriasin, dermcidin, and adrenomedullin, all of which are key components of the innate immune system[23]. These antimicrobial peptides are usually maintained at low levels in healthy skin but are rapidly upregulated during infection or injury, enhancing the skin's antimicrobial defense[24]. NHE, a type of membrane transporter protein, plays a vital role in extracting H+ and regulating Potential of Hydrogen (pH) in the immune system[25]. Healthy skin maintains a slightly acidic pH, which supports the growth of the normal microbiota on the skin's surface, thereby preventing infection and fortifying the epidermal barrier[26]. Impaired NHE1 function raises the pH of the stratum corneum, disrupting lipid metabolism and leading to an immature lamellar structure, which compromises the permeability barrier of the epidermis[27]. Recent studies have shown that antimicrobial peptides like defensins and cathelicidin not only exhibit antimicrobial activity but also activate immune cells such as neutrophils, mast cells, monocytes, and T cells, recruiting them to the site of infection or injury for secondary defense functions[28]. We confirmed that the cathelicidin and NHE positive responses were significantly increased in the BHTG compared to the LBEG and DEXG, suggesting that Baekho-tang may enhance the epidermal barrier by modulating the activity of antimicrobial peptides.

In our experiment, Baekho-tang showed superior effects, particularly in markers related to tight junctions, compared to other groups. While the tight junction markers in other groups decreased, we observed a significant increase in the BHTG. Tight junctions are structures that connect adjacent epidermal cells, controlling the movement of electrolytes and water. Claudin, one of the proteins forming tight junctions, plays an important role in maintaining skin barrier function[29]. It has been reported that claudin-1 and claudin-23 levels are decreased in patients with AD compared to healthy skin, and claudin-1 expression has an inverse relationship with Th2 immune responses[30,31]. Another factor influencing tight junction function is TLR-2, which is reduced in the skin of patients with AD[32]. TLR-2 mediates innate immune responses and strengthens the function of tight junction, although some studies suggest that it may also weaken tight junctions[33]. Additionally, TLR-2 plays a role in recognizing pathogens and activating NF-κB and MAPK pathways, thereby inducing inflammation[34,35]. Since our study showed an increase in TLR-2 positive responses in the BHTG, it suggests the possibility of enhanced tight junction function, though this remains controversial due to conflicting findings.

In clinical KM field, prescriptions containing herbs with heat-clearing properties are often used to suppress inflammatory reactions in various inflammatory diseases[36]. These prescriptions are tailored to individual symptoms and constitutions by adding or modifying herbs accordingly. Baekho-tang, known for its heat-clearing and fluid-generating effects, is suitable for diseases like AD where both inflammation and dryness negatively impact the condition[10]. To date, no studies have reported the effects of Baekho-tang or its constituent herbs, such as gypsum, anemarrhena, licorice, and rice, on the epidermal environment. However, some previous studies have shown that gypsum can modulate inflammation through pathways like TLR-4[37], and ceramides extracted from rice have been reported to increase ceramide levels and improve skin barrier function, suggesting that Baekho-tang may function similarly[38].

In conclusion, Baekho-tang has shown significant effects on certain indicators of inflammation, skin moisturizing factors, antimicrobial barriers, and tight junction function compared to dexamethasone and the untreated condition. Our study highlights positive results related to factors determining the skin epithelial environment in AD. These findings offer promising insights into potential applications for human treatment. In terms of human applications, Baekho-tang could serve as a complementary or alternative therapeutic option for managing inflammatory skin disorders accompanied by damage to the epithelial environment. By enhancing the expression of critical factors involved in skin hydration, antimicrobial defense, and barrier integrity, Baekho-tang provides a multi-faceted approach to skin recovery. Its natural composition may also reduce dependency on conventional treatments like corticosteroids and immunosuppressants, which are often associated with adverse effects. It can be an especially valuable advantage for children, who requires continuous growth and immune system strengthening. However, since the triggers and exacerbating factors of AD are highly complex, further research with more controlled variables would yield stronger evidence. Additionally, this study was conducted on animals, and further research is required before applying these findings to humans.

Ⅴ. Conclusion

In this study, we administered Baekho-tang to an AD-induced animal model and found that it restored moisturizing factors in the skin via ceramide kinase and caspase 14 activity. Additionally, changes in NHE and cathelicidine suggested an enhancement of the antimicrobial barrier. Moreover, we confirmed the potential for strengthening the tight junction function through claudin and TLR-2 activity. The results are summarized as follows:

1. Alleviation of Skin Epithelial Damage

Histological findings showed that, compared to DEXG and LBEG, BHTG significantly reduced dermal edema.

2. Restoration of Skin Moisturizing Factors

1) Ceramide kinase expression increased by 253% compared to LBEG and 93% compared to the control. The increase in BHTG was also higher than in DEXG.
2) Caspase 14 expression increased by 83% compared to the control, and it significantly increased by 23% compared to DEXG.

3. Restoration of Antimicrobial Barrier Function

1) NHE expression increased by 70% compared to the control. NHE expression in BHTG was significantly higher than in LBEG by 151% and DEXG by 40%.
2) Cathelicidine expression decreased in all LBEG, DEXG, and BHTG compared to the control. However, cathelicidine expression in BHTG was significantly higher than LBEG by 184% and DEXG by 47%.

4. Strengthening of Tight Junctions

1) Claudin expression in BHTG increased by 272% compared to LBEG and by 89% compared to DEXG.
2) TLR-2 expression in BHTG increased significantly by 356% compared to LBEG and by 140% compared to DEXG.

Ⅵ. Acknowledgement

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