



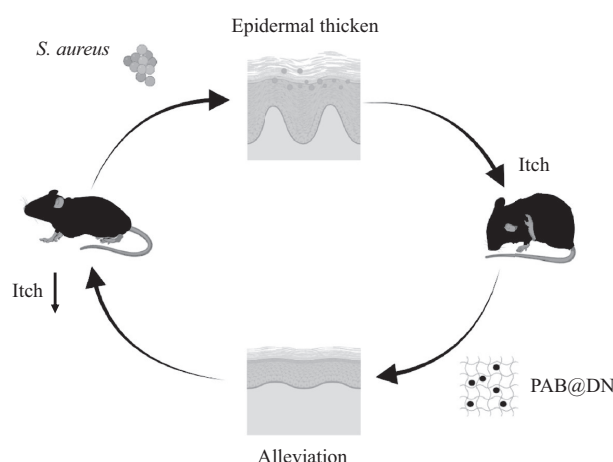
Pseudolaric Acid B-linked Double-network Hydrogel Alleviates Pruritus by Inhibiting The Growth of *Staphylococcus aureus**

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Graphical abstract



Abstract Objective This study aimed to elucidate the mechanistic role of *Staphylococcus aureus* in the pathogenesis of atopic dermatitis (AD), a chronic inflammatory skin disorder characterized by pruritus and barrier dysfunction. A key focus was screening traditional Chinese medicine (TCM) active components with dual antibacterial and antipruritic efficacy, followed by systematic evaluation of their *in vitro* antibacterial activity. Additionally, a novel drug delivery system was constructed to enable localized efficient drug delivery, inhibiting *S. aureus* proliferation and alleviating its induced pruritus, thereby providing new strategies for targeted AD therapy. **Methods** Male C57BL/6J mice aged 6–8 weeks (body weight 18–22 g) were used to establish an AD model via repeated oxazolone sensitization. On day 14, microbial samples were collected from the lesional area (1 cm²) using sterile cotton swabs, followed by vortex mixing, serial dilution, and plating on 5% sheep blood agar plates (incubated at 37°C for 24 h). Single colonies with complete transparent β -hemolytic zones were isolated and identified as vancomycin-intermediate *S. aureus* (VISA) via

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16S rRNA sequencing. An *S. aureus* mono-infection animal model was then established by applying gauze saturated with bacterial suspension (McFarland turbidity 0.1) to the nape and back skin of mice. The pruritic phenotype and inflammatory cell infiltration induced by *S. aureus* were evaluated using comprehensive approaches including behavioral assays (*e.g.*, scratching frequency recording), hematoxylin-eosin (HE) staining, and toluidine blue staining. The *in vitro* antibacterial efficacy of the TCM monomer pseudolaric acid B (PAB) and double network hydrogel (DN) was separately assessed by disk diffusion assay, while the minimum inhibitory concentration (MIC) of PAB was determined *via* broth dilution method. Further validation of the pharmacodynamic characteristics of the composite system (PAB@DN, composed of PAB and DN) was conducted through behavioral assays, HE staining, and dermatitis scoring, with its drug release profile evaluated by mass spectrometry analysis. Based on scratching behavioral analysis and dermatitis scoring, the optimal ratio and concentration of PAB@DN were optimized. **Results** The *S. aureus* load in AD lesional tissues was significantly higher than in normal skin ($(5.3 \pm 0.33) \times 10^6$ CFU *vs.* $(3.6 \pm 0.26) \times 10^7$ CFU, $P < 0.001$). In the *S. aureus* mono-infection group, mice exhibited a 6.7-fold increase in scratching frequency compared to the control group. HE staining revealed marked epidermal thickening ($(10.4 \pm 2.39) \mu\text{m}$ *vs.* $(85.6 \pm 1.95) \mu\text{m}$, $P < 0.0001$), and toluidine blue staining showed a 23-fold increase in mast cell degranulation. Pseudolaric acid B exhibited a significant concentration-dependent inhibitory effect on *S. aureus* growth, with its *in vitro* antibacterial effect being 57% that of the antibiotic cefepime (inhibition zone diameter: PAB (1.885 ± 0.036) cm *vs.* cefepime (3.636 ± 0.005) cm, $P < 0.0001$) and a minimum inhibitory concentration (MIC) of 1 g/L. The carrier double network hydrogel (DN) itself lacked direct antibacterial activity (no significant difference in inhibition zone diameter compared to the control) but effectively ameliorated the dry symptoms of AD-like lesions. The PAB@DN composite system demonstrated a synergistic effect compared to individual components, resulting in a 50% reduction in scratching behavior, an 86% decrease in dermatitis score, and a 60% reduction in epidermal thickening. It also reduced the *S. aureus* load in mouse skin by approximately 34%, with the optimal effective formulation being PAB at 1 g/L loaded onto DN. **Conclusion** *S. aureus* colonization plays a critical driving role in the onset and progression of AD. Using an *S. aureus* infection model, this study confirmed that the pseudolaric acid B-hydrogel composite delivery system (PAB@DN) can effectively alleviate *S. aureus*-induced pruritus and skin damage, providing experimental evidence for microbiota-targeted therapy of AD.

Key words atopic dermatitis, pruritus, *Staphylococcus aureus*, pseudolaric acid B double-network hydrogel

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Atopic dermatitis (AD) is a common chronic inflammatory skin disease characterized by significant global prevalence trends and demographic distribution differences^[1]. Studies have reported that approximately 90% of AD patients show colonization of *Staphylococcus aureus* (*S. aureus*) on their skin^[2], whereas individuals with *S. aureus* infections demonstrate increased susceptibility to developing AD^[3]. *S. aureus* is a prevalent foodborne pathogen that is widely distributed in the environment^[4]. Existing studies have demonstrated that *S. aureus* induces pruritus *via* V8 protease-mediated cleavage of PAR1^[5], thereby elucidating the mechanistic pathway by which bacteria trigger pruritus^[6]. This discovery offers novel insights into potential strategies for alleviating AD-associated pruritus. Current clinical approaches for managing AD-related pruritus mainly rely on antihistamines or corticosteroids^[7-8]. However, these medications are associated with significant adverse effects, including central nervous system

suppression, and rebound phenomena upon discontinuation^[9]. Inspired by these findings, we propose to alleviate AD-associated pruritus by reducing pathogenic bacterial colonization. Antibiotics currently constitute the primary treatment for bacterial infections. However, the precise dosage requirements and pathogen-specific drug selection present challenges for general public use, often leading to antibiotic misuse^[10]. Therefore, this study aims to identify more practical therapeutic alternatives with lower barriers to appropriate use.

The therapeutic effects of traditional Chinese medicine (TCM) have recently attracted increasing attention. Therefore, we screened TCM compounds with antibacterial and antipruritic properties to identify potential candidates for alleviating pruritus in AD. Pseudolarix bark (commonly known as “Tujingpi” or Golden Larch), the dried bark or root bark of *Pseudolarix amabilis* (a member of the Pinaceae family), exhibits potent antibacterial activity

and demonstrates broad application potential^[11]. Pseudolaric acid B (PAB), the primary antibacterial component of Pseudolarix bark, also possesses anti-inflammatory properties. Previous studies have reported that PAB can improve AD-like skin lesions by suppressing interleukin-17 (IL-17)-induced inflammation^[12]. Further studies indicate that PAB downregulates the expression of potent pruritogenic cytokines, including IL-31 and IL-17A^[13], demonstrating significant efficacy in itch relief. In this study, we further confirmed the inhibitory effect on *S. aureus* growth through antibacterial assays.

Pseudolarix bark has traditionally been applied to pruritic skin areas *via* decoction washing^[14-15]. However, this method is limited by a short duration of drug efficacy and inconvenient administration. Hydrogel, as a novel material, plays a significant role in wound management^[16]. An *in situ* sprayable double-network hydrogel (DN) was developed using sodium alginate and chitosan as natural polymeric substrates. This system integrates the hemostatic and immunomodulatory properties of alginate with the pro-migratory and granulation tissue-promoting activities of chitosan, exhibiting excellent biocompatibility and facilitating cell proliferation while reducing apoptosis. Animal studies demonstrated superior wound healing efficacy compared with commercial dressings, evidenced by enhanced collagen maturation and reduced scarring at day 21. Rheological analysis indicated progressive hydrolysis following film formation at 200 s, while human skin testing confirmed a minimum 4-h film durability^[17]. It integrates antibacterial properties with wound healing promotion while adapting to normal skin stretching conditions^[18]. Its three-dimensional porous structure, constructed from a natural polymer network, makes this system very promising as an active drug delivery carrier^[19]. Therefore, this study aims to combine it with PAB to establish a composite delivery system (PAB@DN) for treatment, while repairing the skin barrier, fully exert the pharmacological effects of PAB, achieve sustained drug release, and facilitate administration.

1 Methods and materials

1.1 Animals

Male C57BL/6J mice, 6–8 weeks, were

purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Animals were bred in an environment with 12 h light/dark cycle, free food and water, a temperature of 26°C, and a humidity of 20%. All Animal experiments were conducted under approved protocols and in accordance with the relevant guidelines and regulations of the Institutional Animal Care and Use Committee of Nanjing University of Chinese Medicine (No. 202512A036). All animal experiments also comply with the Guide for The Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

1.2 Atopic dermatitis model

The dorsal cervical and abdominal hair of mice was removed two days prior. The mice were pre-sensitized on the abdomen with 2% oxazolone (diluted in glycerol) for 2 d, followed by induction on the dorsal skin with 0.5% oxazolone. In this study, male mice were used in the experiments.

1.3 Isolation and purification of *S. aureus* colonies from the dorsal skin of AD mice

A sterilized cotton swab moistened with sterilized PBS was rubbed back and forth over a 1 cm² area of the modeled AD mouse skin, then immersed in 1 ml of PBS. After vortex mixing, the solution was diluted 100-fold, and 50 µl was spread on a blood agar plate. Colonies with hemolytic zones were selected for streak plate isolation and purification.

1.4 *S. aureus* infection model

An overnight culture of *S. aureus* was diluted to a McFarland standard of 0.1. A 1 cm² gauze was soaked in the bacterial suspension and applied to the pre-shaved dorsal cervical skin of the mice. A transparent dressing was used to secure the gauze and prevent detachment. After 24 h, the gauze and dressing were removed, and a 30-min video recording was conducted to quantify scratching episodes, recorded as Day 1. After recording, the gauze was re-soaked in the bacterial suspension and reapplied. Behavioral changes were recorded every 24 h for a total of 5 d.

1.5 Antibacterial zone experiment

A bacterial suspension with a McFarland standard of 0.1 was diluted 1 000-fold, and 50 µl was evenly spread on Luria-Bertani medium. 7 mm diameter wells were punched into the medium using a

200 μ l pipette tip. Then, 30 μ l of the test drug solution was added to each well. The plate was incubated at 37° C for 24 h, after which the diameter of the inhibition zones was measured.

1.6 Broth microdilution method

A bacterial suspension with a McFarland standard of 0.1 was diluted 2 000-fold for plating. The test drug solution was serially diluted, with six replicate wells set for each concentration. In a 96-well plate, 200 μ l of the drug-bacteria mixture was added to each well, while the peripheral wells were filled with blank medium. The plate was incubated at 37° C for 24 h. After incubation, the A_{600} was measured using a microplate reader, and the results were statistically analyzed.

1.7 Minimum inhibitory concentration (MIC)

A bacterial suspension with a McFarland standard of 0.1 was diluted 1 000-fold. The diluted bacterial suspension was used to prepare the highest test concentration of the drug solution, which was then serially diluted with the bacterial suspension. Mixtures of bacteria and drug at different concentrations were added to culture tubes and incubated on a shaker at 37° C. After 24 h, the tubes were examined for clarity. The concentration of the tube that appeared clear to the naked eye was recorded as the MIC.

1.8 Synthesis of PAB@DN hydrogel

The sprayed hydrogel consists from two kinds of solutions. One composed with sodium alginate and sodium carboxymethyl cellulose. The solution was under magnetic stirring at 60° C until it becoming clear and uniform. The other solution was prepared with acetic acid solution, chitosan, CaCl_2 . The preparation process of hydrogel membrane was to spray the two types of solution onto the surface of substrates. Hydrogel membrane will be formed in the first 10 s.

1.9 Drug release

500 μ l of PBS was gently added to 500 μ l of drug-loaded gel. The supernatant (10 μ l) was collected at 0.5, 1, 2, 4, 8, 12, and 24 h for analysis by high-performance liquid chromatography (HPLC).

1.10 Skin colony quantification

An 8 mm² section of skin from the modeled or treated dorsal cervical area of the mice was collected and placed in cold PBS. After homogenization, the

sample was serially diluted, spread on Luria-Bertani medium, and incubated for 24 h for colony counting.

1.11 Statistical analysis

All experiments were conducted in triplicate, as independent experiments. Statistical analyses were performed using GraphPad Prism software. Results are presented as mean $\pm$ SEM. Data were statistically analyzed with a two-tailed, paired/unpaired Student's *t*-test and two-way ANOVA. For all, $P < 0.05$ was considered statistically significant.

2 Results

2.1 *S. aureus* isolated from AD mice induced pruritus

To confirm the colonization of *S. aureus* (SA) on AD-affected skin, microbial analyses were performed on dorsal skin swabs obtained from AD model mice (Figure 1a). Hemolytic halos were observed on blood agar plates inoculated with these samples (Figure 1b). Subsequent isolation, purification, and 16S rRNA gene sequencing identified these colonies as Vancomycin-Intermediate *Staphylococcus aureus* (VISA), which was used for subsequent experimental modeling. To establish causality, mice were subsequently infected with the isolated *S. aureus* strain. The results indicated that *S. aureus*-infected mice exhibited more severe dermatitis symptoms compared to standard AD model mice on the fifth day (Figure 1c). Behavioral analysis revealed a significant increase in scratching behavior in infected mice compared to control mice ($n=3$; Day1: Control (11.3 $\pm$ 5.78) times, AD (3.3 $\pm$ 1.202) times, VISA (155.3 $\pm$ 11.724) times, Day3: Control (13.7 $\pm$ 2.667) times; AD (38.3 $\pm$ 0.882) times; VISA (128 $\pm$ 1.155) times, Day5: Control (14.7 $\pm$ 6.227) times; AD (93.3 $\pm$ 4.410) times, VISA (101 $\pm$ 11.150) times) (Figure 1d). Collectively, these findings confirm that *S. aureus* colonization on AD skin actively induces significant pruritus.

2.2 *S. aureus* infection induces chronic pruritus and epidermal thickening

To further investigate the role of *S. aureus* in chronic pruritus, a 5-d bacterial infection model was established^[5] in mice (Figure 2a). The results indicated that infected mice exhibited a significant increase in scratching behavior compared to the control group ($P < 0.01$, $n=4$) (Figure 2b). Histopathological examination with hematoxylin and eosin staining (HE staining) revealed obvious

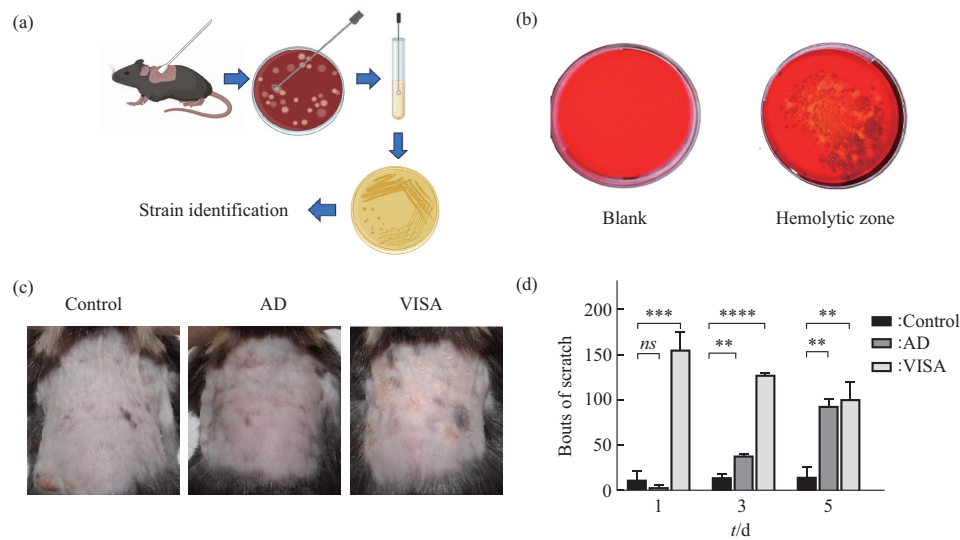


Fig. 1 *S. aureus* from atopic dermatitis mice induced significant pruritus

(a) Flowchart of colony screening and identification from swabs of the dorsal skin of atopic dermatitis mice. (b) Colonies showing hemolytic zones on blood agar plates. (c) Comparison of skin conditions between mice modeled with atopic dermatitis and *Staphylococcus aureus*. (d) Statistical analysis of scratching behavior in mice modeled with atopic dermatitis and *Staphylococcus aureus* ($n=3$). ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$, ns: not significant. AD: atopic dermatitis; VISA: Vancomycin Intermediate *Staphylococcus aureus*.

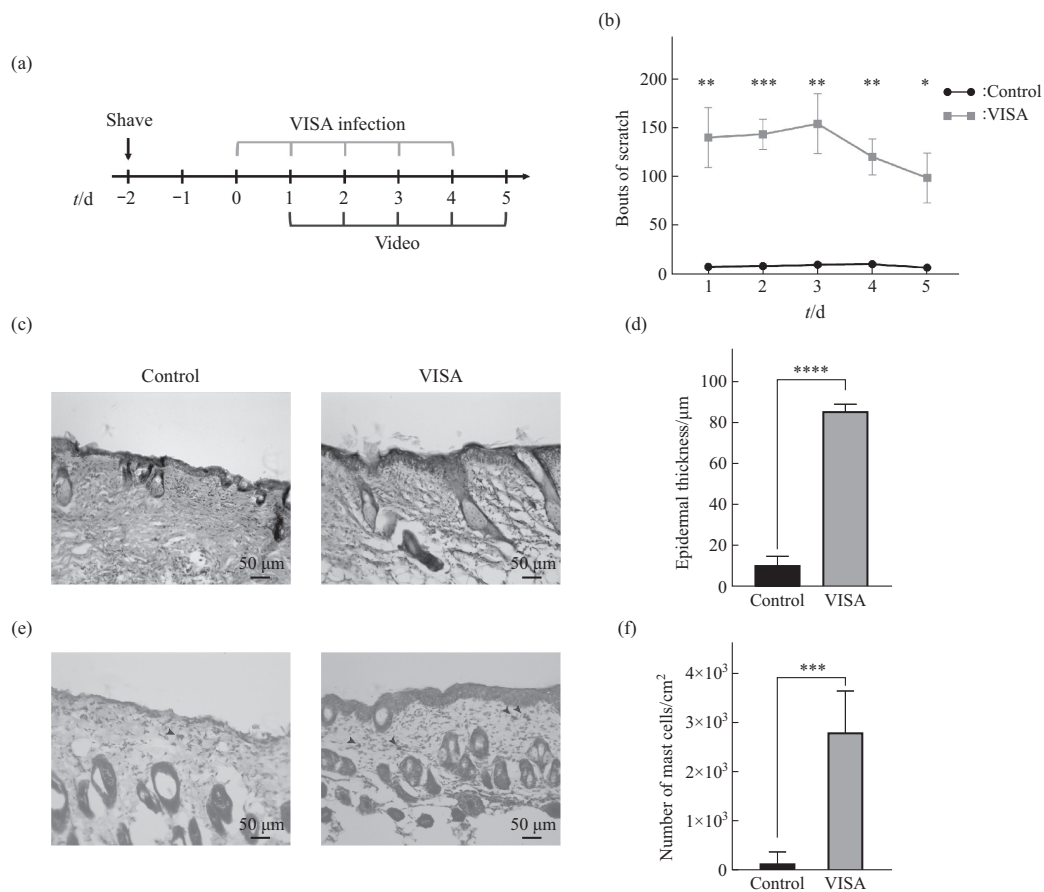


Fig. 2 *Staphylococcus aureus* infection induced typical atopic dermatitis-like symptoms

(a) Schematic diagram of establishing the *Staphylococcus aureus* infection model in mice. (b) Significant increase in scratching behavior in mice after dorsal skin infection with *Staphylococcus aureus* ($n=4$). (c) Obvious epidermal thickening in mice after modeling ($n=4$). (d) Statistical results showing significant differences ($n=4$). (e) Schematic diagram of toluidine blue staining ($n=4$). (f) Statistical results of mast cell numbers ($n=4$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$. VISA: Vancomycin Intermediate *Staphylococcus aureus*.

epidermal thickening in the infected mice, with statistically significant differences ($P < 0.001$, $n = 3$, Control (10.4 ± 2.390) μm vs. VISA (85.6 ± 1.95) μm) (Figure 2c, d). The number of mast cells was also significantly higher in infected mice than in control mice (Figure 2e, f). These findings demonstrate that *S. aureus* skin infection induces severe chronic pruritus and inflammatory responses.

2.3 Pseudolaric acid B significantly inhibits the growth of *S. aureus*

Pseudolarix bark, widely utilized in traditional Chinese medicine (TCM) for its insecticidal and antipruritic properties, contains PAB as its principal active component, which has been reported to possess

significant antimicrobial activity. To evaluate the inhibitory effects of PAB on the growth of *S. aureus*, cefepime was employed as a positive control, and antibacterial efficacy was assessed. Compared to the control group, PAB exhibited a highly significant inhibitory effect, as indicated by the diameter of the inhibition zone ($P < 0.0001$, $n = 3$, Control (0.703 ± 0.009) cm; Hydrogel (0.71 ± 0.006) cm, PAB (1.885 ± 0.036) cm, Cefepime (3.636 ± 0.005) cm) (Figure 3a, b). Further testing using the broth dilution method revealed that the antibacterial activity of PAB was concentration-dependent (Figure 3c). The MIC was determined to be 1 g/L (Figure 3d). These results confirm that PAB can significantly inhibit the growth of *S. aureus*.

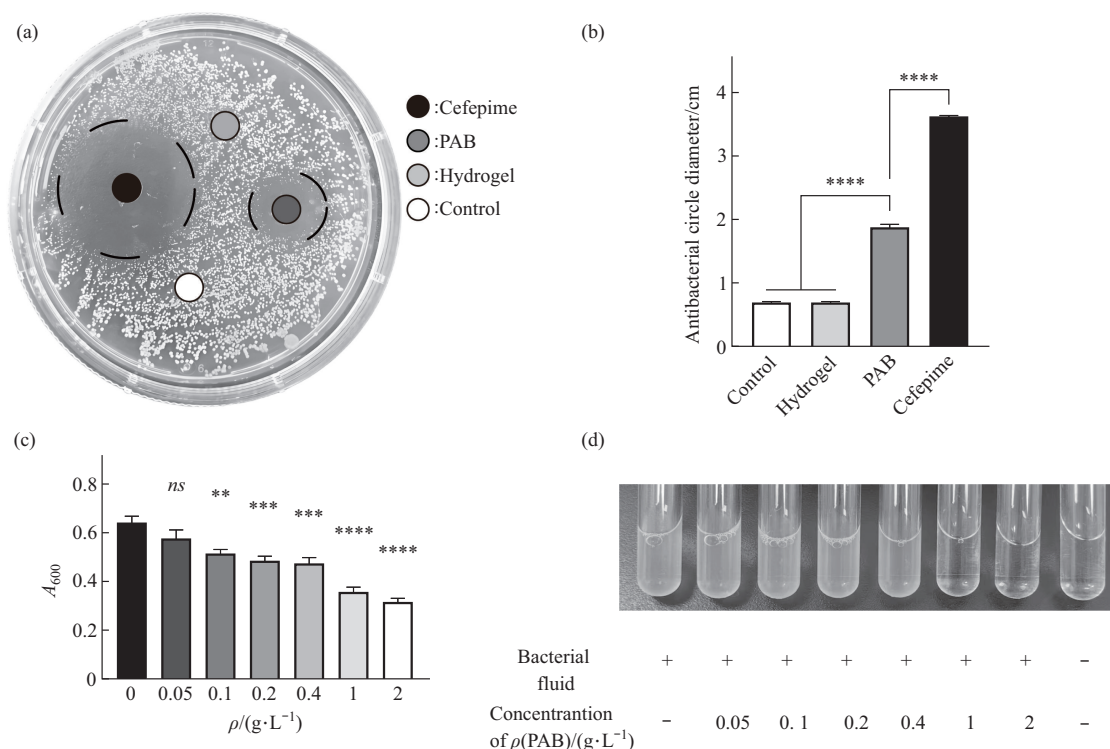


Fig. 3 Pseudolaric acid B (PAB) exhibits significant antibacterial effects

(a) Antibacterial zone experiment demonstrates strong antibacterial activity of pseudolaric acid B (PAB). The dotted circle represents the antibacterial zone. (b) Statistical results of inhibition zones show significant differences ($n = 4$). (c) Antibacterial effect of pseudolaric acid B exhibits concentration-dependent gradient ($n = 6$). (d) The minimum inhibitory concentration (MIC) of PAB is 1 g/L. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ns: not significant.

2.4 PAB combined with double-network hydrogel (PAB@DN) significantly alleviates *S. aureus*-induced pruritus and inflammation

To identify an optimal carrier for the active component PAB and enhance its therapeutic efficacy^[16], concentration-dependent experiments

were conducted. The MIC of PAB was determined to be 1 g/L. To evaluate the enhanced efficacy of PAB-hydrogel combination, drug release kinetics were measured (Figure 4a), revealing a peak PAB release of 150 mg/L at 4 h post-administration. For therapeutic assessment, five experimental groups were

established: infection model controls, blank hydrogel-treated, 150 mg/L PAB-treated, 1 g/L PAB-treated, and 1 g/L PAB@DN-treated mice. Behavioral analysis of scratching and dermatitis scoring demonstrated that, although the blank hydrogel did not significantly affect scratching behavior ($P < 0.05$, $n = 4$, Control (12.8 ± 3.146) times; VISA (155.8 ± 16.245) times; VISA+Blank hydrogel (145 ± 11.295) times; VISA+150 mg/L PAB (117.8 ± 23.3) times; VISA+1 g/L PAB (73 ± 7.533) times; VISA+1 g/L PAB@DN (68.7 ± 6.96) times) (Figure 4b), it showed a tendency to improve dermatitis scores and significantly reduced epidermal thickening ($P < 0.05$, $n = 4$, Control (10.3 ± 2.433) μm ; VISA (63.1 ± 5.964) μm ; VISA+Blank hydrogel (40.4 ± 4.894) μm ; VISA+150 mg/L PAB (37.6 ± 0.931) μm ; VISA+1 g/L PAB (22.8 ± 1.386) μm ; VISA+1 g/L PAB@DN (20.5 ± 0.872) μm) (Figure 4c–e). PAB monotherapy groups

exhibited significant reductions in both pruritic behavior and skin hyperplasia. Notably, the PAB@DN group showed superior performance, with more pronounced suppression of scratching behavior, greater improvement in skin thickening, and better dermatitis scores compared to either treatment alone (Figure 4b–e). Microbiological analysis of dorsal skin samples^[13] confirmed a significant reduction in *S. aureus* colonization following combination therapy ($P < 0.01$, $n = 3$, Control ($5.3 \times 10^6 \pm 3.33 \times 10^5$) CFU; VISA ($3.6 \times 10^7 \pm 2.6 \times 10^6$) CFU; VISA+1 g/L PAB@DN ($1.4 \times 10^7 \pm 3.2 \times 10^6$) CFU) (Figure 4f). Collectively, these findings demonstrate that the PAB@DN system effectively inhibits *S. aureus* proliferation, thereby alleviating infection-induced pruritus and improving cutaneous pathology.

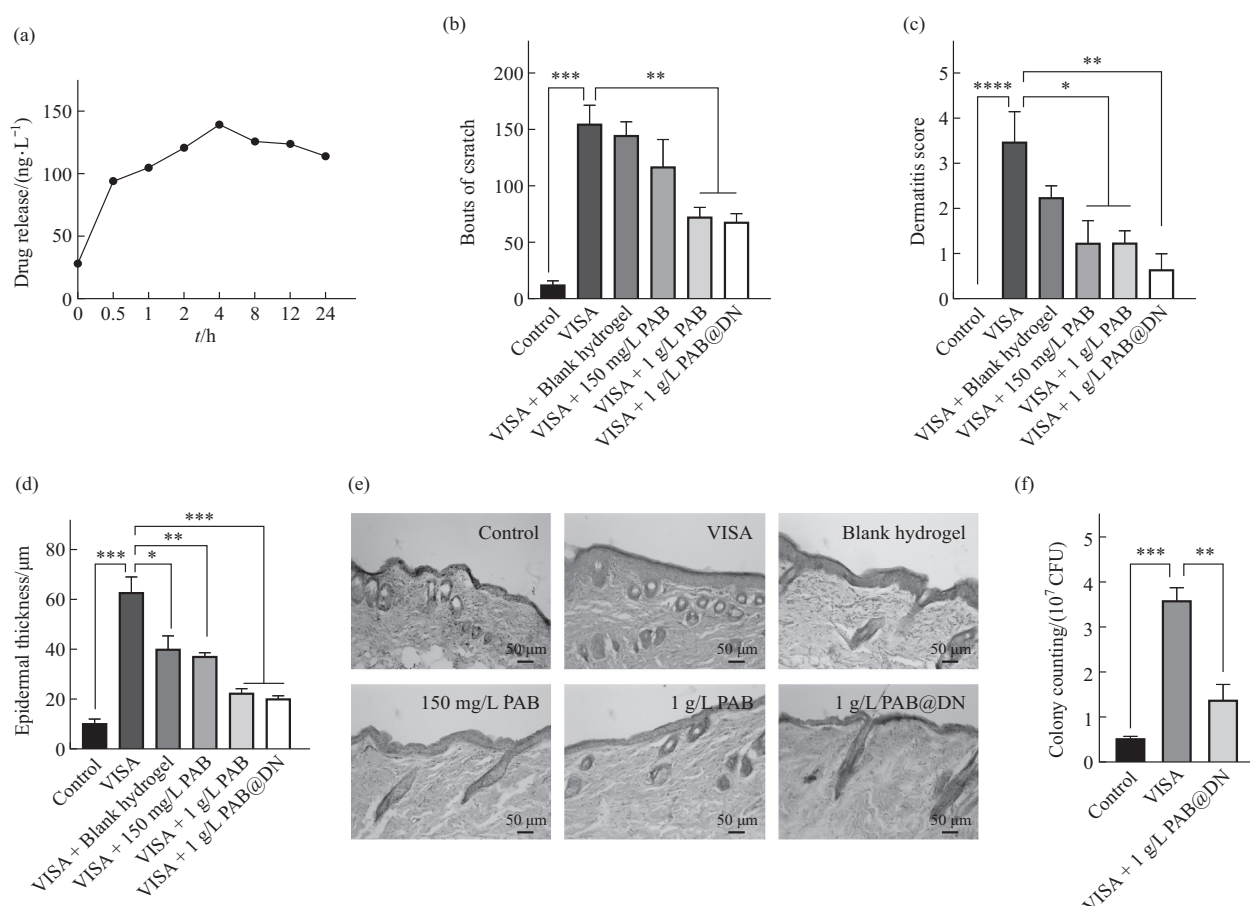


Fig. 4 Combined drug delivery achieved sustained release and effective treatment

(a) Drug release amount of pseudolactic acid B mixed with hydrogel peaks at 4 h, approximately 150 mg/L. (b–d) *Staphylococcus aureus*-infected mice (VISA) were treated with blank hydrogel, 150 mg/L pseudolactic acid B (PAB), 1 g/L pseudolactic acid B, and a mixture of 1 g/L pseudolactic acid B with hydrogel, respectively. Scratching behavior, dermatitis scores, and epidermal thickening were observed. (e) HE staining of skin from different treatment groups. (f) Biopsy samples of 8 mm² dorsal skin from mice were homogenized, serially diluted, and plated for colony counting ($n = 3$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

2.5 1 g/L PAB@DN demonstrated optimal therapeutic efficacy

Our previous study confirmed the optimal therapeutic effect of PAB@DN system. To further determine the most effective concentration, we tested low, medium and high doses of PAB in hydrogel formulations. The results demonstrated that the 1 g/L PAB@DN showed the most favorable therapeutic outcomes in both scratching behavior and dermatitis scores (Figure 5a, b). Detailed examination of four

dermatitis parameters (erythema, welt, desquamation and dryness) revealed that 1 g/L PAB@DN particularly exhibited significant improvements in erythema and dryness ($P < 0.05$, $n = 3$) (Figure 5c, d). Although all tested PAB concentrations significantly alleviated scratching behavior and inflammation ($P < 0.05$, $n = 3$), the 1 g/L PAB@DN showed superior effects in maintaining wound hydration and reducing inflammation ($P < 0.05$, $n = 3$), confirming it as the optimal concentration for treatment.

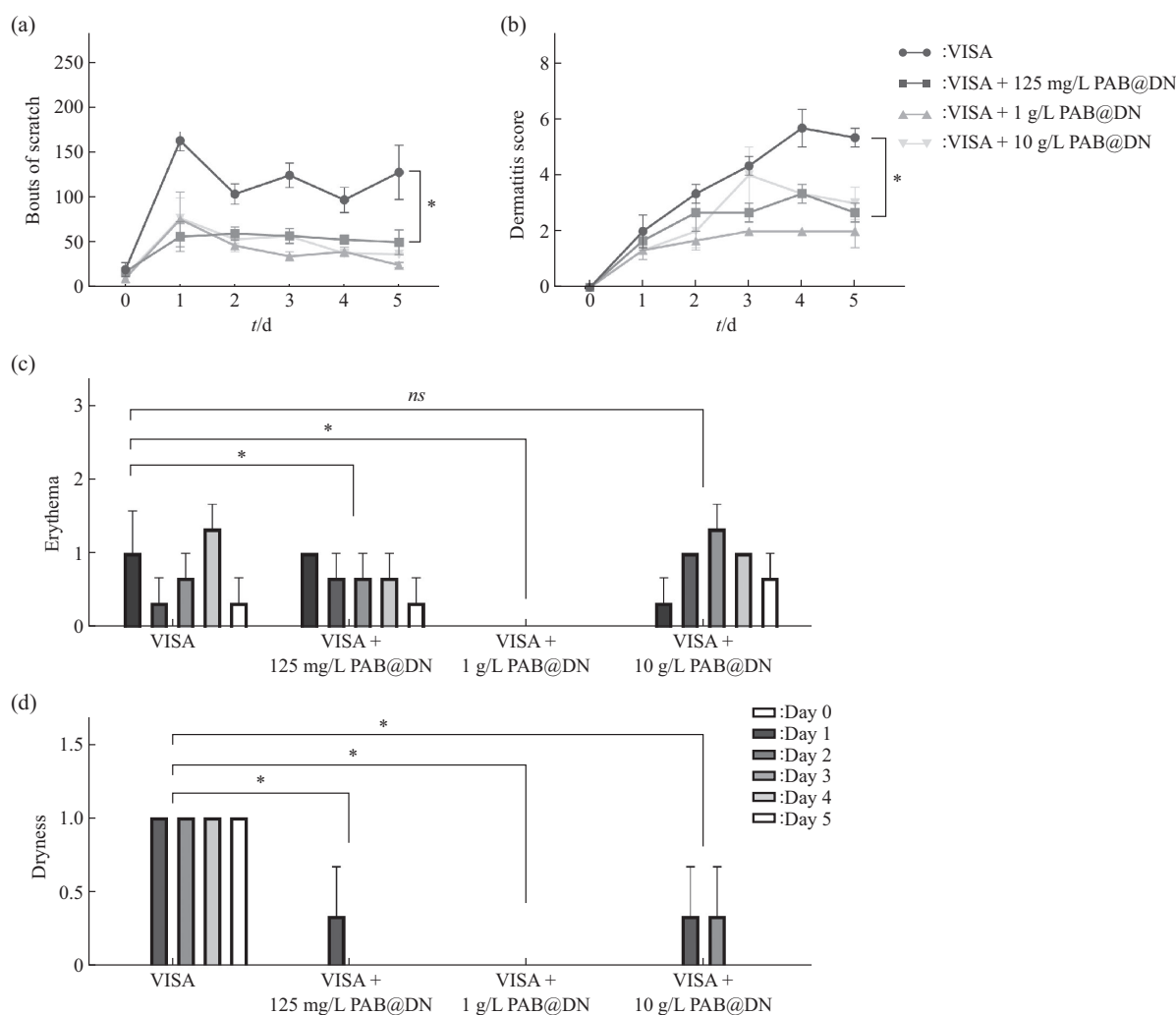


Fig.5 Optimal concentration of combined drug delivery determined through *in vivo* experiments

(a, b) Low, medium, and high doses of PAB@DN were administered, and scratching behavior and dermatitis scores were observed ($n = 3$). (c, d) Individual analysis of the four dermatitis score indicators: erythema and dryness. ($n = 3$). * $P < 0.05$, ns: not significant. VISA: Vancomycin Intermediate *Staphylococcus aureus*; PAB@DN: pseudolaric acid B linked double-network hydrogel.

3 Discussion

Colonization and infection of *S. aureus* on the skin can induce and perpetuate chronic pruritus. This

finding demonstrates a close association between microbial dysbiosis and the pathological progression of chronic inflammatory skin diseases, such as AD^[5]. Moreover, it provides a theoretical foundation for

managing AD-associated pruritus by modulating the skin microbiome and intervening in microbe-host interactions. Although antibiotics are frequently used to treat *S. aureus* infections, they are often associated with adverse effects, such as drug resistance, allergic reactions, and disruption of the gut microbiota^[20]. Traditional Chinese herbal medicines not only exhibit significant antibacterial properties but also demonstrate immunomodulatory effects and protective benefits for intestinal microbial ecology.

PAB, a bioactive monomer derived from traditional Chinese medicine, exhibits well-characterized anti-inflammatory and antimicrobial activities, thereby partially overcoming the limitations associated with conventional therapeutics, such as corticosteroids, in long-term prophylactic use. However, studies have shown that high doses of PAB may induce gastrointestinal irritation and central neurotoxicity^[21], necessitating careful optimization of both the administration route and dosage. To avoid gastrointestinal toxicity, topical administration was employed. Furthermore, a hydrogel was utilized as a drug carrier, capitalizing on its hydrophilic network structure to facilitate sustained drug release^[22], extend local therapeutic effects, and improve bioavailability. Additionally, the hydrogel matrix forms a physical barrier, substantially reducing direct irritation of the skin and mucous membranes caused by free drug molecules. Finally, by adjusting the pH of the drug-loaded hydrogel to near-neutral levels, potential secondary damage to the compromised skin barrier in AD was minimized. The drug concentration was determined according to the conventional range of existing topical formulations and supplemented by exploratory studies to balance efficacy and safety.

Currently, research specifically targeting the molecular mechanism of PAB against *S. aureus* remains very limited. PAB belongs to the diterpenoid family of compounds. Based on diterpenoid structural characteristics and established antibacterial mechanisms, we postulate that PAB exerts bactericidal effects through two primary pathways: cytoplasmic membrane disruption^[23-24] and biofilm inhibition^[25]. The former leads to intracellular content leakage and bacterial death, while the latter attenuates bacterial colonization and enhances susceptibility to host defenses and antimicrobial agents. Despite a higher MIC against *S. aureus* (1 g/L) compared to conventional antibiotics, PAB offers distinct

advantages through its multi-target synergistic effects in the treatment of AD. Beyond antibacterial effects, PAB exerts anti-inflammatory and immunomodulatory actions^[12, 26], suppresses pathological angiogenesis^[27] and keratinocyte hyperproliferation^[12], and promotes metabolic regulation^[28] and barrier repair^[29]. This multi-target mechanism reduces resistance risk while addressing the complex pathological network of AD, positioning PAB as a safe and effective natural therapeutic candidate.

Beyond its prominent antibacterial activity, PAB exhibits well-defined dermatitis-reparative effects. Mechanistically, PAB suppresses T cell activation and macrophage M1 polarization through the inhibition of NF- κ B signaling and p38 MAPK phosphorylation, thereby attenuating inflammatory responses^[30]. Additionally, PAB ameliorates IL-17-induced inflammatory reactions via modulation of the PPAR γ /NF- κ B/IL-17 axis, resulting in reduced serum IgE levels and decreased inflammatory cell infiltration. These actions collectively contribute to significant improvement of AD-like skin lesions in NC/Nga mice and promotion of dermatitis repair^[12].

In summary, this study developed a hydrogel-based topical formulation of PAB with optimized physicochemical properties (*e. g.*, pH), providing a preliminary approach to reduce systemic toxicity and local irritation. The results suggest that PAB, particularly in its optimized topical form, holds potential for AD management, especially in preventing *S. aureus*-induced pruritus recurrence and maintaining remission. Nevertheless, certain limitations persist, including long-term local safety^[31] (*e. g.*, skin irritation and sensitization), transdermal absorption efficiency, and pharmacokinetic profiles of the hydrogel system. Future research will further elucidate the molecular mechanisms by which PAB modulates *S. aureus*-host interactions in the cutaneous microenvironment and systematically evaluate its preclinical pharmacodynamics and safety to facilitate clinical translation.

4 Conclusion

Using an *S. aureus* mono-infection model, this study demonstrated that administration of the PAB@DN composite system significantly reduced the

S. aureus load in the lesional area, decreased scratching behavior, and alleviated epidermal thickening in mice with *S. aureus* infection. The system exhibits triple synergistic effects of targeted antibacterial action, anti-pruritus, and skin barrier protection, thereby providing a novel safe and effective candidate strategy for the clinical treatment of AD.

Data Availability The data related to this paper (DOI: 10.57760/sciencedb.32872/CSTR: 31253.11.sciencedb.32872) can be accessed from the Science Data Bank database (<https://www.scidb.cn/anonymous/cUViRTdy>).

References

- [1] Tian J, Zhang D, Yang Y, *et al.* Global epidemiology of atopic dermatitis: a comprehensive systematic analysis and modelling study. *Br J Dermatol*, 2023, **190**(1): 55-61
- [2] Gallo R L, Horswill A R. *Staphylococcus aureus*: the bug behind the itch in atopic dermatitis. *J Invest Dermatol*, 2024, **144**(5): 950-953
- [3] Nakatsuji T, Chen T H, Two A M, *et al.* *Staphylococcus aureus* exploits epidermal barrier defects in atopic dermatitis to trigger cytokine expression. *J Invest Dermatol*, 2016, **136**(11): 2192-2200
- [4] Chalmers S J, Wylam M E. Methicillin-resistant *Staphylococcus aureus* infection and treatment options. *Methods Mol Biol*, 2020, **2069**: 229-251
- [5] Deng L, Costa F, Blake K J, *et al.* *S. aureus* drives itch and scratch-induced skin damage through a V8 protease-PAR1 axis. *Cell*, 2023, **186**(24): 5375-5393.e25
- [6] Nakamura Y, Oscherwitz J, Cease K B, *et al.* *Staphylococcus* δ -toxin induces allergic skin disease by activating mast cells. *Nature*, 2013, **503**(7476): 397-401
- [7] Hur M S, Choe Y B, Ahn K J, *et al.* Synergistic effect of H1-antihistamines on topical corticosteroids for pruritus in atopic dermatitis: a systematic review and meta-analysis. *Ann Dermatol*, 2019, **31**(4): 420-425
- [8] Chu D K, Chu A W L, Rayner D G, *et al.* Topical treatments for atopic dermatitis (eczema): systematic review and network meta-analysis of randomized trials. *J Allergy Clin Immunol*, 2023, **152**(6): 1493-1519
- [9] Yanai K, Yoshikawa T, Church M K. Efficacy and safety of non-brain penetrating H₁-antihistamines for the treatment of allergic diseases. *Curr Top Behav Neurosci*, 2022, **59**: 193-214
- [10] Mithuna R, Tharanyalakshmi R, Jain I, *et al.* Emergence of antibiotic resistance due to the excessive use of antibiotics in medicines and feed additives: a global scenario with emphasis on the Indian perspective. *Emerg Contam*, 2024, **10**(4): 100389
- [11] Yin M, Li N, Zhang L, *et al.* Pseudolaric acid B ameliorates fungal keratitis progression by suppressing inflammation and reducing fungal load. *ACS Infect Dis*, 2023, **9**(6): 1196-1205
- [12] Yang Z, Liu M, Wang W, *et al.* Pseudolaric acid B attenuates atopic dermatitis-like skin lesions by inhibiting interleukin-17-induced inflammation. *Sci Rep*, 2017, **7**(1): 7918
- [13] Terui H, Yamasaki K, Wada-Irimada M, *et al.* *Staphylococcus aureus* skin colonization promotes SLE-like autoimmune inflammation via neutrophil activation and the IL-23/IL-17 axis. *Sci Immunol*, 2022, **7**(76): eabm9811
- [14] Han Q B, Yip Y K, Yang N Y, *et al.* Rapid analysis of pseudolaric acids in Cortex Pseudolaricis and related medicinal products by high performance liquid chromatography. *Talanta*, 2007, **73**(4): 757-763
- [15] Zhang J, Yan L T, Yuan E L, *et al.* Antifungal activity of compounds extracted from Cortex Pseudolaricis against *Colletotrichum gloeosporioides*. *J Agric Food Chem*, 2014, **62**(21): 4905-4910
- [16] Zhang A, Liu Y, Qin D, *et al.* Research status of self-healing hydrogel for wound management: a review. *Int J Biol Macromol*, 2020, **164**: 2108-2123
- [17] Cai C, Wang T, Han X, *et al.* *In situ* wound sprayable double-network hydrogel: preparation and characterization. *Chin Chem Lett*, 2022, **33**(4): 1963-1969
- [18] Arabpour Z, Abedi F, Salehi M, *et al.* Hydrogel-based skin regeneration. *Int J Mol Sci*, 2024, **25**(4): 1982
- [19] Ding H, Liu R, Ruan H, *et al.* Rapidly gelling sprayable hydrogels integrating carbon quantum dots and plant extracellular vesicles enable multifunctional wound care via photothermal management, immunomodulation, and ferroptosis. *Small*, 2025, **21**(35): e2503202
- [20] Chen L, Li A, Li J H, *et al.* Mechanism of action of lacticaseibacillus paracasei HNU502 and fecal microbiota transplantation in alleviating antibiotic side effects. *Food Sci*, 2025, **46**(3): 1-10
- [21] Xu Q, Deng H, Huang X, *et al.* Design, synthesis, and *in vivo* and *in vitro* biological screening of pseudolaric acid B derivatives as potential anti-tumor agents. *Bioorg Chem*, 2024, **151**: 107670
- [22] Jia Y, Hu J, An K, *et al.* Hydrogel dressing integrating FAK inhibition and ROS scavenging for mechano-chemical treatment of atopic dermatitis. *Nat Commun*, 2023, **14**(1): 2478
- [23] Tan L, Yang J, He Z, *et al.* Inhibitory effects of extracts from *Prunella vulgaris* on biofilm formation of *Staphylococcus aureus*. *Microb Pathog*, 2025, **205**: 107694
- [24] Merghni A, Belmamoun A R, Urcan A C, *et al.* 1, 8-cineol (eucalyptol) disrupts membrane integrity and induces oxidative stress in methicillin-resistant *Staphylococcus aureus*. *Antioxidants (Basel)*, 2023, **12**(7): 1388
- [25] Kumar G, Engle K. Natural products acting against *S. aureus* through membrane and cell wall disruption. *Nat Prod Rep*, 2023, **40**(10): 1608-1646
- [26] Lu J, Guan H, Wu D, *et al.* Pseudolaric acid B ameliorates synovial inflammation and vessel formation by stabilizing PPAR γ to inhibit NF- κ B signalling pathway. *J Cell Mol Med*, 2021, **25**(14): 6664-6678

- [27] Li M H, Miao Z H, Tan W F, *et al.* Pseudolaric acid B inhibits angiogenesis and reduces hypoxia-inducible factor 1 alpha by promoting proteasome-mediated degradation. Clin Cancer Res, 2004, **10**(24): 8266-8274
- [28] Liu S Y, Zhang X W, Gao G, *et al.* Pseudolaric acid B alleviates non-alcoholic fatty liver disease by targeting PPAR α to regulate lipid metabolism and promote mitochondrial biogenesis. Chin J Integr Med, 2025, **31**(10): 877-888
- [29] Li X, Zhao X, Song W, *et al.* Pseudolaric acid B inhibits proliferation, invasion and epithelial-to-mesenchymal transition in human pancreatic cancer cell. Yonsei Med J, 2018, **59**(1): 20-27
- [30] Li Y, Li T, Ji W, *et al.* Inhibitory effects of pseudolaric acid B on inflammatory response and M1 phenotype polarization in RAW_{264.7} macrophages induced by lipopolysaccharide. Chin J Cell Mol Immunol, 2016, **32**(5): 625-629
- [31] Wen C, Chen J, Zhang D, *et al.* Pseudolaric acid B induces mitotic arrest and apoptosis in both 5-fluorouracil-sensitive and-resistant colorectal cancer cells. Cancer Lett, 2016, **383**(2): 295-308

土荆皮乙酸交联双网络水凝胶通过抑制金黄色葡萄球菌缓解瘙痒*

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摘要 目的 金黄色葡萄球菌 (*Staphylococcus aureus*) 的大量定植是加重特应性皮炎 (atopic dermatitis, AD) 瘙痒及皮损的重要因素。本研究通过建立金黄色葡萄球菌感染小鼠模型, 阐明金黄色葡萄球菌在AD中的致痒作用, 并探讨土荆皮乙酸 (PAB) 混合双网水凝胶 (DN) 水凝胶新型载药体系 (PAB@DN) 对金黄色葡萄球菌诱导瘙痒的改善效应。**方法** 分离AD小鼠皮损部位定植的金黄色葡萄球菌, 并构建金黄色葡萄球菌单独感染模型, 明确金黄色葡萄球菌的致痒作用。通过体外抑菌实验检测土荆皮乙酸的抑菌效果及抑菌浓度, 构建PAB@DN缓释给药系统, 评估其抗菌止痒, 皮肤修复作用。**结果** 金黄色葡萄球菌感染可引起小鼠搔抓行为显著升高, 呈现AD样皮损。PAB@DN缓释给药后, 模型小鼠搔抓次数明显减少, 表皮增厚缓解, 皮损区 *S.aureus* 载量较模型组降低34%。**结论** 本研究通过金黄色葡萄球菌单一感染模型, 明确土荆皮乙酸混合水凝胶缓释给药可以明显缓解金黄色葡萄球菌引起的瘙痒及皮肤损伤。

关键词 特应性皮炎, 瘙痒, 金黄色葡萄球菌, 土荆皮乙酸双网络水凝胶

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