Diospyros lotus leaf extract and its main component, myricitrin, inhibit both histamine-dependent and histamine-independent itching

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Received February 18, 2025; Accepted April 4, 2025

DOI: 10.3892/etm.2025.12871

Abstract. Pruritus is a distressing symptom associated with various dermatological, systemic and neurological conditions, markedly impairing quality of life. Pruritus arises through histamine-dependent and histamine-independent pathways, involving mediators such as histamine, gastrin-releasing peptide (GRP), interleukin-31 (IL-31) and STAT3 signaling. The present study aimed to investigate the antipruritic effects of *Diospyros lotus* leaf extract (DLE) and its major constituent, myricitrin (MC), on ICR mice using compound 48/80 (histamine-dependent) and chloroquine (histamine-independent) itch models. Serum levels of histamine and IL-31 were measured by ELISA, and mast cell infiltration was assessed via toluidine blue staining. Furthermore, the expression and activation of GRP receptor (GRPR), IL-31RA and STAT3 in the spinal cord were analyzed using western blotting and immunofluorescence staining. Notably, DLE and MC significantly reduced scratching behavior, serum histamine levels and mast cell infiltration in both models. Immunofluorescence staining and western blot analysis revealed that DLE and MC downregulated GRPR, IL-31 receptor A and phosphorylated STAT3 expression in the spinal cord, indicating modulation of central itch signaling. Additionally, DLE and MC suppressed IL-31 levels in serum and skin tissues. These findings indicated that DLE and MC may alleviate pruritus through multiple mechanisms, including mast cell stabilization, histamine reduction and modulation of central itch pathways. The broad-spectrum antipruritic activity of DLE and MC highlights their potential as natural therapeutic agents for diverse pruritic conditions, offering a safer alternative to synthetic antipruritic drugs. Further research is warranted to validate these findings in clinical settings and to elucidate the molecular mechanisms underlying their efficacy.

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Key words: pruritus, *Diospyros lotus*, myricitrin, interleukin-31, STAT3 signaling, antipruritic effects

Introduction

Pruritus, commonly referred to as itching, is a complex sensory phenomenon characterized by an irresistible urge to scratch in response to stimuli affecting the skin or mucous membranes (1). As a prevalent and often debilitating symptom, pruritus is associated with a wide range of dermatological, systemic, and neurological disorders, significantly impairing the quality of life for affected individuals (2). Itching can present as either localized or generalized and varies in intensity from mild to severe, depending on the underlying etiology (3). The pathogenesis of pruritus is multifactorial, involving a combination of intrinsic and extrinsic factors. Intrinsic factors include chronic infections, circulatory disorders, endocrine and metabolic dysfunctions, and genetic predispositions to allergic reactions, while extrinsic triggers encompass environmental agents such as food allergens, airborne particles, chemical irritants, and animal-derived allergens (4,5).

The mechanisms underlying pruritus are broadly categorized into histamine-dependent and histamine-independent pathways. Histamine, a well-known pruritogen, is primarily released by mast cells and is predominantly associated with acute itching (6). Mast cell activation, mediated by IgE, lipopolysaccharides (LPS), cytokines, and chemokines, leads to degranulation and the subsequent release of itch-inducing mediators, including histamine, interleukin-4 (IL-4), and interleukin-31 (IL-31) (7). Compound 48/80, a synthetic mast cell activator, has been widely used in experimental studies to investigate histamine-mediated itch pathways due to its ability to induce mast cell degranulation and histamine release via the histamine-1 receptor (H1R) (8,9). In contrast, chronic itching is often histamine-independent and is associated with severe or recurrent pathological conditions (10). Non-histaminergic pruriceptive neurons can be activated by

various endogenous and exogenous pruritogens, such as proteases, cytokines, chemokines, and amines, which interact with specific receptors on sensory neurons (11). Recent advances in understanding the neural circuitry of itch have highlighted the role of gastrin-releasing peptide (GRP) and its receptor (GRPR) in the spinal dorsal horn. GRP, released from primary itch afferents, activates GRPR-expressing neurons in lamina I of the dorsal horn, playing a critical role in itch signal transmission (12-14). Chloroquine, an antimalarial drug, is a notable example of a histamine-independent pruritogen that induces severe itching through the GRP/GRPR signaling pathway, independent of histamine (15,16). This property makes chloroquine a valuable tool for studying histamine-independent itch mechanisms.

Diospyros lotus, a perennial woody plant of the *Ebenaceae* family, is native to various regions in Asia, including Korea (17). Extracts from *D. lotus* leaves (DLE) are rich in polyphenolic compounds, including gallic acid, caffeic acid, chlorogenic acid, myricetin-3-O-galactoside, myricitrin (MC), astragalin, quercetin, and myricetin, with myricitrin being the most abundant, at approximately 86 μg/mg (18,19). DLE has demonstrated a range of pharmacological properties, including anti-obesity, anti-photoaging, and hepatoprotective effects, attributed to its high polyphenol content (19,20). Additionally, DLE has shown anti-pruritic effects in various animal models, including atopic dermatitis induced by 2,4-dinitrofluorobenzene (DNFB) and house dust mite antigens, as well as acute itch models induced by compound 48/80 and chloroquine (21,22). While existing research has primarily focused on the cutaneous effects of DLE, its potential impact on the central nervous system remains underexplored.

Given this context, the present study aims to investigate the effects of DLE and its major constituent, myricitrin, on both histamine-dependent and histamine-independent itch pathways in ICR mice. Specifically, we will evaluate their efficacy in alleviating pruritus induced by compound 48/80 (histamine-dependent) and chloroquine (histamine-independent), thereby providing insights into the potential mechanisms of action of DLE and MC in alleviating pruritus.

Materials and methods

Plant material collection and preparation. Fresh leaves of *D. lotus* were collected in June 2022 from Cheonjam Mountain, Jeonju-si, Jeollabuk-do, Republic of Korea. The plant species was identified and authenticated by Professor Hong-Jun Kim from the College of Oriental Medicine at Woosuk University, Jeonbuk, Republic of Korea. A voucher specimen (#2022-06-04) was deposited in the Department of Health Management, College of Medical Science, Jeonju University, for future reference. The collected leaves were thoroughly washed with distilled water to remove impurities and dried in a well-ventilated, shaded area to prevent degradation of bioactive compounds. Dried leaves (100 g) were extracted with 2 l of 70% (v/v) ethanol at room temperature for 48 h. The extract was filtered twice through a 0.5-μm membrane filter (ADVANTEC), concentrated under vacuum (EYELA Rotary evaporator N-1100, EYELA), and freeze-dried to obtain a powdered form of *D. lotus* leaf extract (DLE).

Reagents and chemicals. Toluidine blue (198161), chloroquine diphosphate (C-271), and compound 48/80 (C2313) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Myricitrin (M2361) was obtained from Tokyo Chemical Industry (Tokyo, Japan). A histamine ELISA kit (ENZ-KIT140A-0001) was procured from Enzo Life Sciences (Farmingdale, NY, USA). Primary antibodies against GRPR (sc-398549), IL-31RA (sc-515465), p-STAT3 (sc-81523), STAT3 (sc-8019), m-IgGκ BP-HRP (sc-516102) and β-actin (sc-8432) were acquired from Santa Cruz Biotechnology (Dallas, TX, USA). An IL-31 ELISA kit (ab213872) was sourced from Abcam (Cambridge, UK). RIPA buffer (89900), goat anti-mouse IgG Alexa Fluor 488 secondary antibody (A-11001) and goat anti-rabbit IgG HRP secondary antibody (31460) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). WestGlow FEMTO (BWF0100) from Biomax (Guri, Korea).

Animals and experimental design. Male ICR mice (4 weeks old, specific-pathogen-free) were purchased from Orient Bio Inc. (Gwangju, South Korea). The mice were housed under controlled environmental conditions (temperature: 22±2˚C, humidity: 50-60%, 12-h light/dark cycle) with *ad libitum* access to a standard laboratory diet and water. The present study was approved by Jeonju University Institutional Animal Care and Use Committee. The health status of the animals was monitored daily, and humane experimental endpoints were established as a 20% or greater loss of body weight, decreased appetite for more than two consecutive days, dyspnea, increased heart rate, self-mutilation, jaundice, persistent diarrhea or vomiting, or a decreased response to external stimuli. No mortality or euthanasia occurred during the study. After a 1-week acclimatization period, the dorsal hair of the mice was shaved using an electric clipper. The mice were randomly divided into seven groups (n=5 per group): i) Normal control (saline), ii) compound 48/80 alone, iii) compound 48/80 + 200 mg/kg DLE, iv) compound 48/80 + 20 mg/kg MC, v) chloroquine alone, vi) chloroquine + 200 mg/kg DLE, and vii) chloroquine + 20 mg/kg MC. The dose of DLE (200 mg/kg) was selected based on previous studies (18-22) that evaluated its physiological activities, including anti-itch, anti-inflammatory, and other bioactive properties. These studies identified 200 mg/kg as an effective concentration. A previous study reported that DLE contains approximately 86 μg of MC per mg of extract (18). Based on this, administering 200 mg/kg of DLE corresponds to an estimated MC intake of approximately 17 mg/kg. To account for variability in MC content and measurement, the MC dose was set at 20 mg/kg. At the end of the experiment, mice were anesthetized with a 2-6% isoflurane for induction and maintained at a 1-3% concentration. Blood samples (600-800 μl per animal, collected once) were collected from the orbital venous plexus. Subsequently, mice were euthanized through cervical dislocation and, after confirming the absence of respiratory and heartbeat, the dorsal skin and vertebral tissues were harvested and either 4% paraformaldehyde preserve in formalin or deep frozen at -80˚C for further analysis.

Scratching behavior analysis. One day prior to the experiment, the dorsal hair of the mice was shaved using a hair clipper. A total of 1 h before the administration of compound 48/80 or chloroquine, mice in groups 1 and 2 received oral saline, while mice in groups 3 to 7 were orally administered either

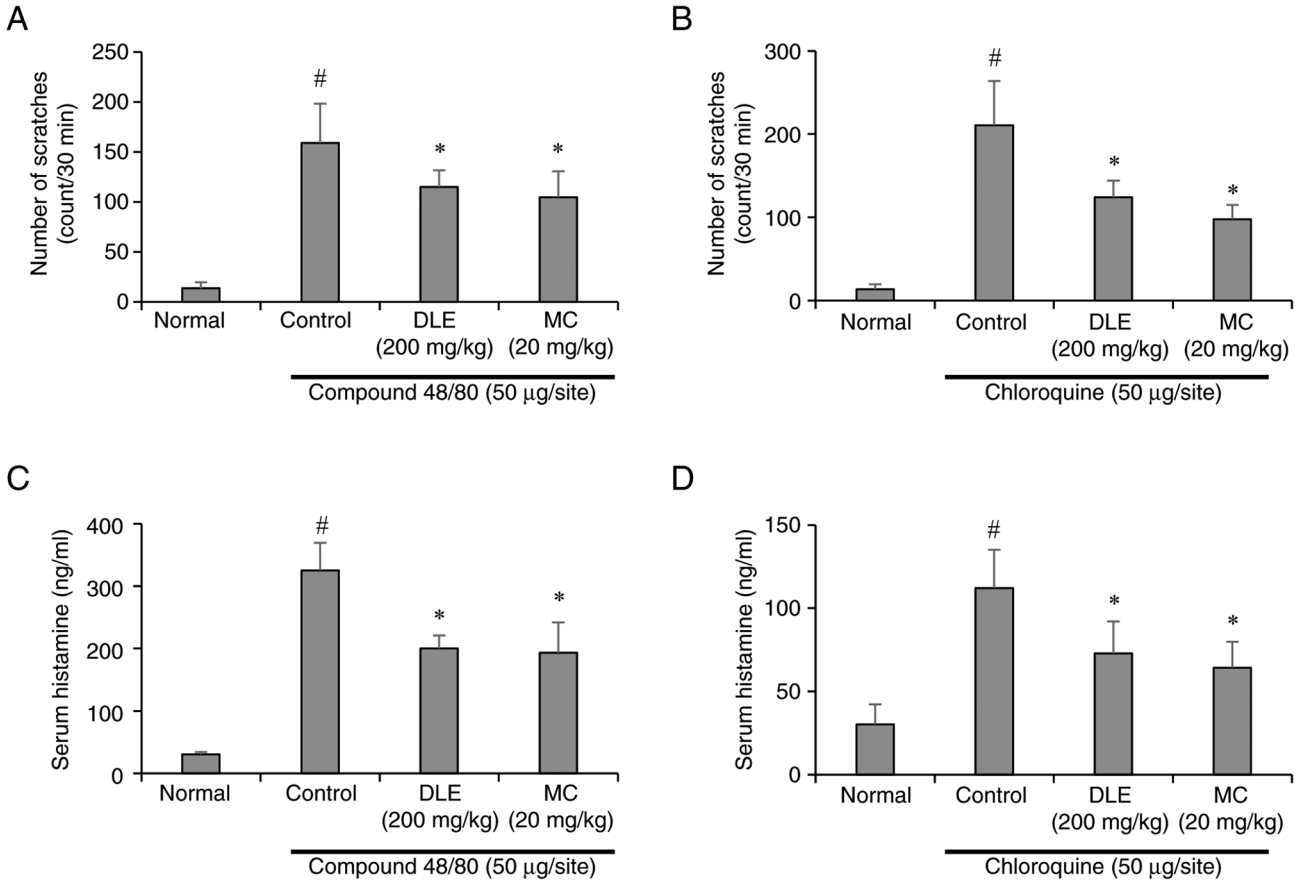

Figure 1. Effects of DLE and MC on scratching behavior and serum histamine levels in compound 48/80 or chloroquine-injected ICR mice. (A) Scratching behavior for 30 min in the compound 48/80-injected group. (B) Scratching behavior for 30 min in the chloroquine-injected group. (C) Serum histamine levels in the compound 48/80-injected group. (D) Serum histamine levels in the chloroquine-injected group. Counting of scratching behavior was conducted through the double-blinded method. Each bar represents the mean ± SD (n=5). $^{\#}$P<0.05 vs. normal group. *P<0.05 vs. control group. DLE, *D. lotus* leaf extract; MC, myricitrin.

DLE or MC. Scratching behavior was video-recorded for 30 min immediately after the injection of compound 48/80 or chloroquine. The recordings were analyzed in a double-blind manner by independent researchers to minimize bias. After the behavioral assessment, blood and dorsal skin samples were collected for histopathological analysis, and vertebral tissues were isolated for immunofluorescence staining.

Histopathological examination. Dorsal skin tissues were fixed in 4% paraformaldehyde for 24 h at 4˚C, followed by washing in phosphate-buffered saline (PBS) with five changes (30 min each). Tissues were dehydrated in a graded ethanol series (60% to 100%) for 30 min per concentration, cleared in xylene (two changes, 2 h each), and embedded in paraffin (three changes, 1 h each). Paraffin-embedded tissues were sectioned at 5 μm thickness using a microtome (Leica, Wetzlar, Germany). Tissue sections were stained with toluidine blue to evaluate mast cell infiltration and degranulation.

Immunofluorescence staining. A segment of the 4-5th lumbar spine was fixed in 4% paraformaldehyde for 4 h at room temperature and then incubated in PBS containing 30% sucrose at 4˚C overnight. Tissues were sectioned at 30 μm thickness using a Cryotome (Amos Scientific, Clayton South, Australia). Sections were washed three times in PBS (10 min each) and incubated for 1 h in PBS containing 0.3% Triton X-100 and 2% Bovine Serum Albumin (BSA). Tissues were then incubated overnight at 4˚C with primary antibodies against GRPR (1:100), IL-31RA (1:100), and p-STAT3 (1:50). After five washes in PBS, sections were incubated with FITC-conjugated goat anti-rat IgG secondary antibodies for 2 h at room temperature. Following additional washes, tissues were mounted with DAPI-containing mounting medium and visualized under a ZEISS fluorescence microscope (Oberkochen, Germany).

Protein extraction and western blotting. Segments of the 4th-5th lumbar spinal cord or dorsal skin were homogenized in RIPA buffer containing protease and phosphatase inhibitors. The homogenate was centrifuged at 12,000 x g for 15 min at 4˚C, and the supernatant was collected to remove debris. Protein concentration was determined using the Bradford assay, and 50 μg of protein was loaded per lane. Samples were separated by SDS-PAGE on a 12% or 7.5% gel and transferred onto a polyvinylidene fluoride (PVDF) membrane. Membranes were blocked with 5% skim milk at room temperature for 1 h and then washed five times with TBST (5 min per wash). Membranes were incubated overnight at 4˚C with the following primary antibodies: GRPR (1:500), IL-31RA (1:500), STAT3 (1:1,000), p-STAT3 (1:500), IL-31 (1:2,000), and β-actin (1:500). After five washes with TBST, membranes

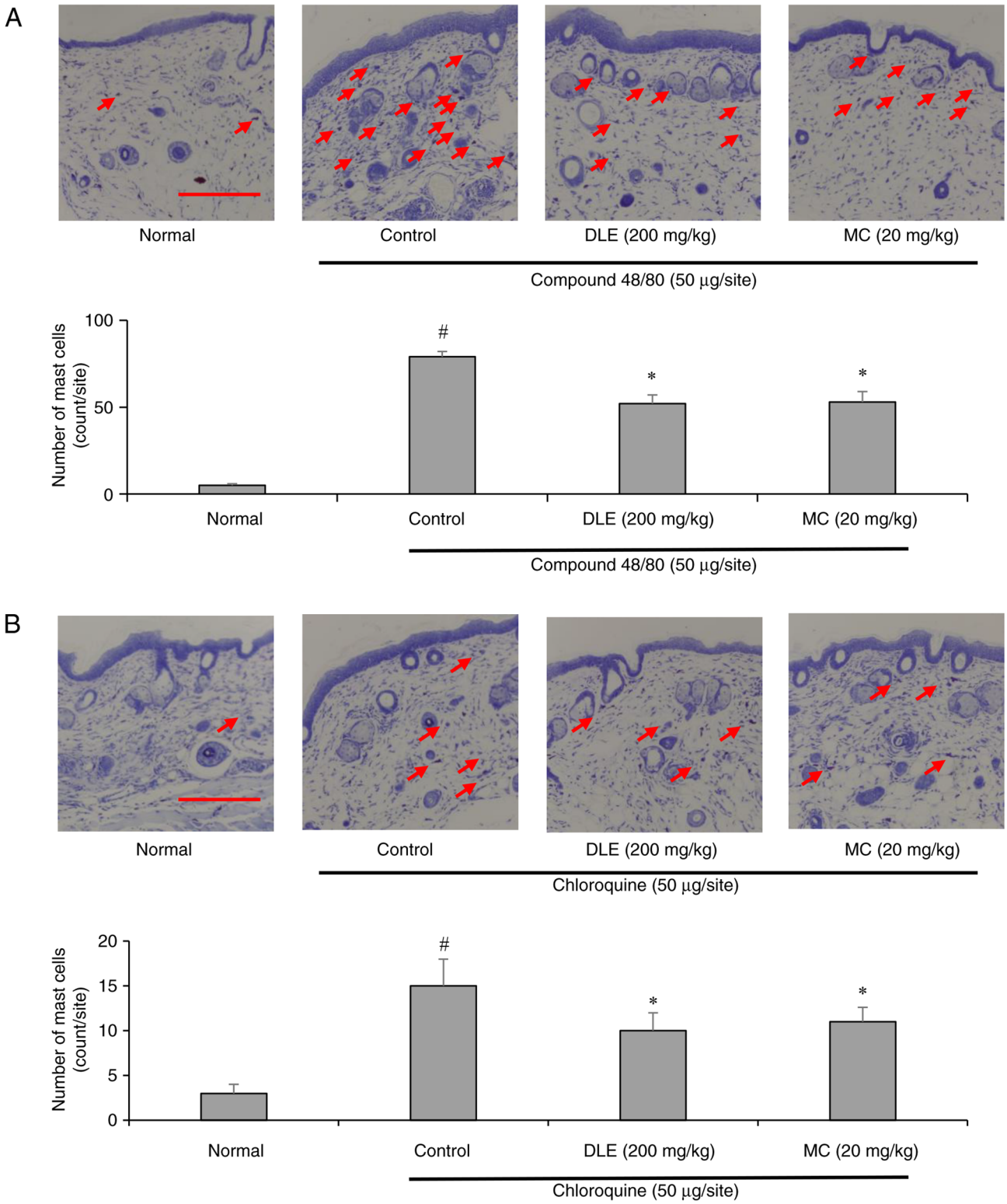

Figure 2. Effects of DLE and MC on mast cell infiltration in compound 48/80 or chloroquine-injected ICR mice. Representative toluidine blue staining images of skin with red arrows indicating stained mast cells (scale bar, 200 μm). The graph shows the number of mast cells counted in mice injected with (A) compound 48/80 and (B) chloroquine. Each bar represents the mean ± SD (n=5). $^{\#}$P<0.05 vs. normal group. *P<0.05 vs. control group. DLE, *D. lotus* leaf extract; MC, myricitrin.

were incubated with HRP-conjugated secondary antibodies in 5% skim milk for 2 h at room temperature. Following five additional washes with TBST, protein bands were visualized using the ALLIANCE LD4 imaging system (Uvitec, Cambridge, UK) with WestGlow FEMTO chemiluminescent reagent. Band densities were analyzed using ImageJ 1.53e (National Institutes of Health), with β-actin used as the loading control.

Statistical analysis. All statistical analysis was performed using SPSS version 26.0 (IBM, Armonk, NY, USA). Data are

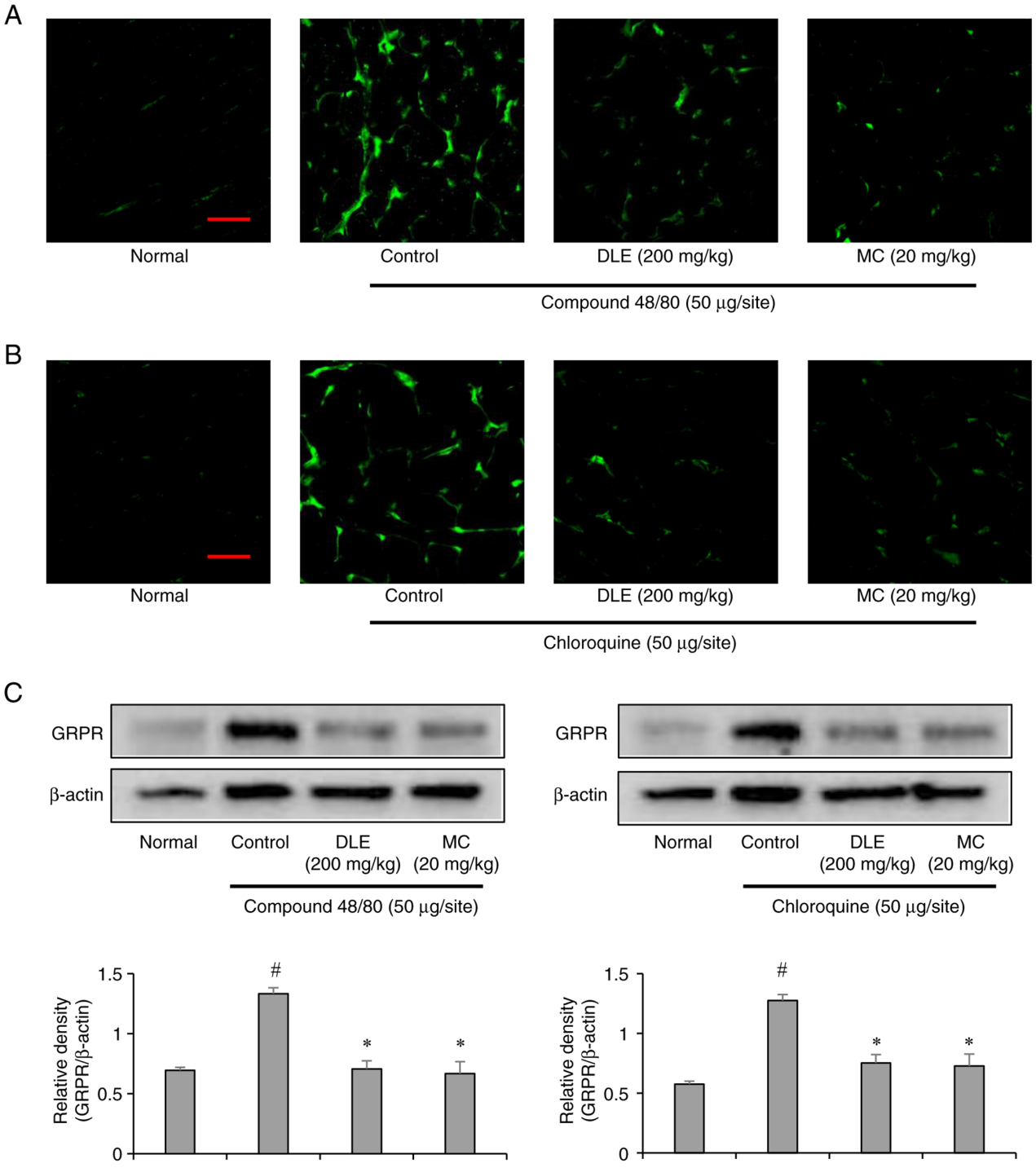

Figure 3. Effects of DLE and MC on spinal cord GRPR in compound 48/80 or chloroquine-injected ICR mice. Spinal cord immunofluorescence stained with antibodies against GRPR (green) in mice injected with (A) compound 48/80 and (B) chloroquine (scale bar, 40 μm). (C) Western blotting data showing the expression of GRPR in the spinal cord tissue for both compound 48/80 and chloroquine-injected mice. Each bar represents the mean ± SD (n=5). #P<0.05 vs. normal group. *P<0.05 vs. control group. DLE, *D. lotus* leaf extract; MC, myricitrin; GRPR, gastrin-releasing peptide receptor.

presented as the mean ± standard deviation (n=5). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Antipruritic effects of DLE and MC on compound 48/80 or chloroquine-induced pruritus. To evaluate the antipruritic effects of DLE and its major constituent, MC, scratching behavior was assessed in ICR mice following subcutaneous injection of compound 48/80 or chloroquine. Both pruritogens significantly increased scratching behavior compared to the normal control group, indicating successful induction of itch. However, pretreatment with DLE (200 mg/kg) or MC (20 mg/kg) significantly attenuated scratching behavior in both compound 48/80- and chloroquine-induced models (Fig. 1A and B). These findings suggest that DLE and MC exhibit significant antipruritic

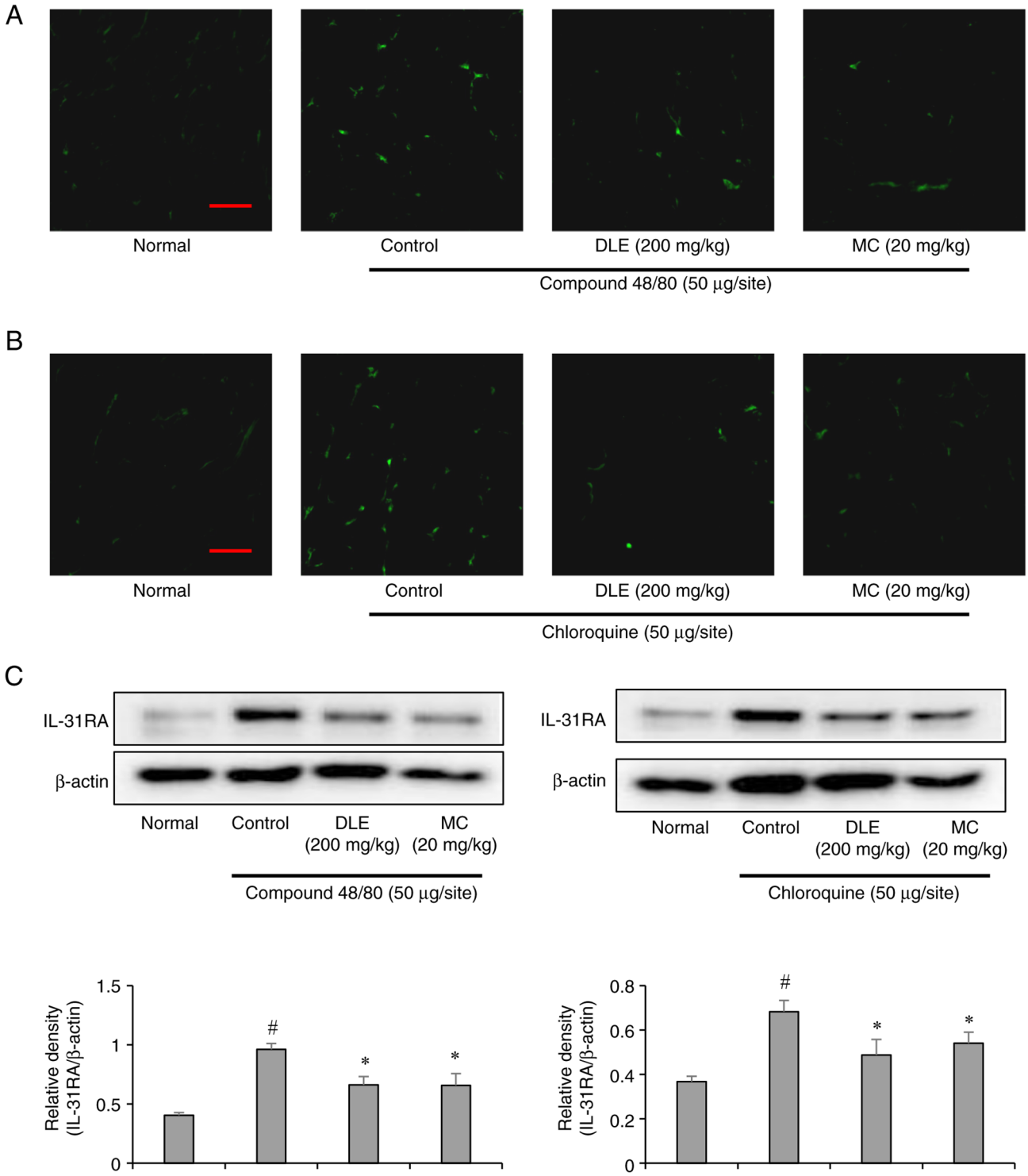

Figure 4. Effects of DLE and MC on spinal cord IL-31RA in compound 48/80 or chloroquine-injected ICR mice. Spinal cord immunofluorescence stained with antibodies against IL-31RA (green) in mice injected with (A) compound 48/80 and (B) chloroquine (scale bar, 40 μm). (C) Western blotting data showing the expression of IL-31RA in the spinal cord tissue for both compound 48/80 and chloroquine-injected mice. Each bar represents the mean ± SD (n=5). $^{\#}$P<0.05 vs. normal group. *P<0.05 vs. control group. DLE, *D. lotus* leaf extract; MC, myricitrin; IL-31RA, interleukin-31 receptor A.

properties against both histamine-dependent and histamine-independent itch pathways. To further elucidate the mechanisms underlying these effects, serum histamine levels were measured using ELISA. Compound 48/80, a known mast cell degranulator, induced a marked increase in serum histamine levels, consistent with its histamine-dependent pruritic action. In contrast, chloroquine, which operates through histamine-independent pathways, caused a comparatively smaller increase in histamine levels. Notably, administration of DLE and MC significantly reduced serum histamine levels in both models (Fig. 1C and D), indicating their ability to modulate mast cell activity and histamine release.

Effects of DLE and MC on mast cell infiltration in compound 48/80- or chloroquine-induced pruritus. Mast

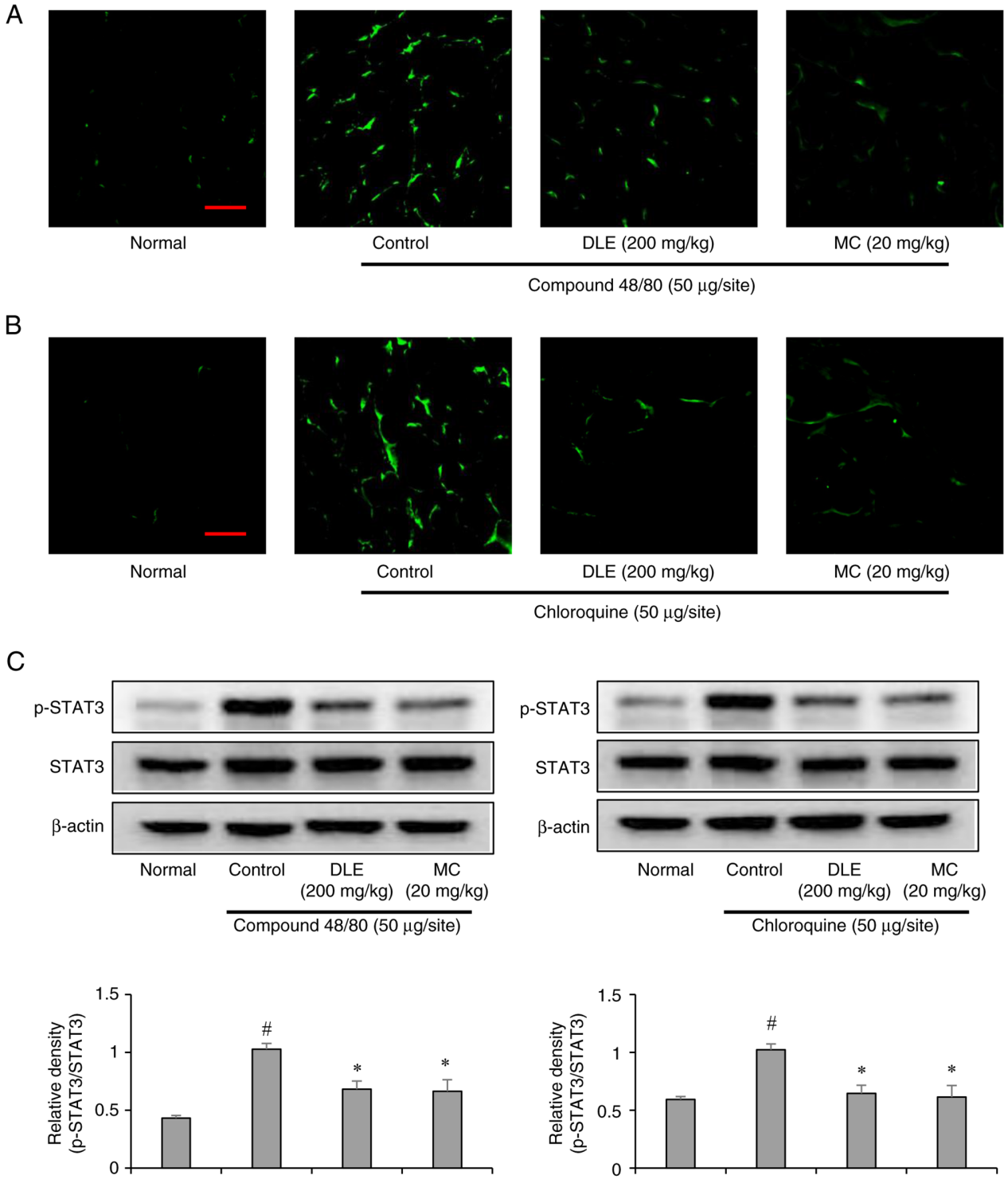

Figure 5. Effects of DLE and MC on spinal cord STAT3 in compound 48/80 or chloroquine-injected ICR mice. Spinal cord immunofluorescence stained with antibodies against STAT3 (green) in mice injected with (A) compound 48/80 and (B) chloroquine (scale bar, 40 μm). (C) Western blotting data showing the expression of STAT3 in the spinal cord tissue for both compound 48/80 and chloroquine-injected mice. Each bar represents the mean ± SD (n=5). $^{\#}$P<0.05 vs. normal group. *P<0.05 vs. control group. DLE, *D. lotus* leaf extract; MC, myricitrin; p-, phosphorylated.

cells play a pivotal role in itch pathogenesis, particularly in histamine-dependent pruritus (23). To assess the impact of DLE and MC on mast cell infiltration, toluidine blue staining was performed on dorsal skin tissues. Compound 48/80 injection resulted in a significant increase in mast cell infiltration, whereas chloroquine induced a milder but still notable increase. Treatment with DLE and MC markedly reduced mast cell infiltration in both models (Fig. 2A and B), suggesting that these compounds inhibit mast cell recruitment and activation, thereby mitigating pruritus.

Effects of DLE and MC on spinal cord GRPR expression. Gastrin-releasing peptide receptor (GRPR) is a key neurotransmitter involved in itch signal transmission within the spinal

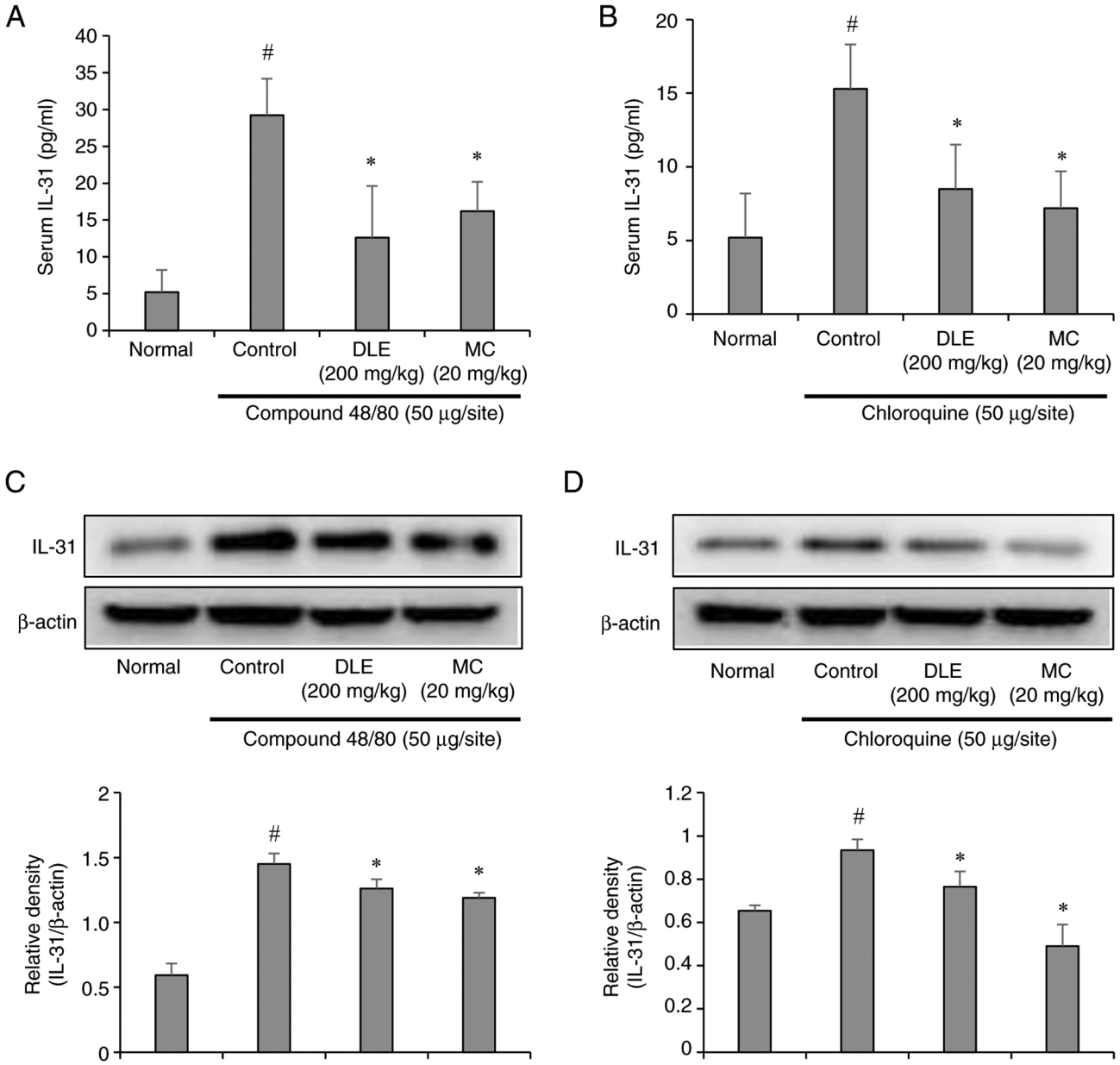

Figure 6. Effects of DLE and MC on serum and skin IL-31 levels in compound 48/80 or chloroquine-injected ICR mice. (A) Serum IL-31 levels in the compound 48/80-injected group. (B) Serum IL-31 levels in the chloroquine-injected group. (C) IL-31 expression in dorsal skin tissue of compound 48/80-injected group. (D) IL-31 expression in dorsal skin tissue of chloroquine-injected group. IL-31 levels were measured using ELISA and western blot. Each bar represents the mean ± SD (n=5). $^{\#}$P<0.05 vs. normal group. *P<0.05 vs. control group. DLE, *D. lotus* leaf extract; MC, myricitrin; IL-31, interleukin-31.

cord (24). Immunofluorescence staining of the lumbar spinal cord (L4-L5) revealed elevated GRPR expression in both compound 48/80- and chloroquine-induced groups, consistent with their pruritogenic effects. However, pretreatment with DLE and MC significantly suppressed GRPR expression in both models (Fig. 3A-C). These results suggest that DLE and MC modulate central itch signaling pathways by downregulating GRPR expression, thereby reducing itch sensation.

Effects of DLE and MC on spinal cord IL-31RA expression. Interleukin-31 receptor A (IL-31RA) is a critical mediator of chronic itch and neuroimmune interactions (25). Immunofluorescence analysis demonstrated increased IL-31RA expression in the spinal cord of mice injected with compound 48/80 or chloroquine. However, treatment with DLE and MC significantly attenuated IL-31RA expression in both groups (Fig. 4A-C). These findings indicate that DLE and MC may alleviate pruritus by modulating IL-31 signaling pathways, which are implicated in chronic itch conditions.

Effects of DLE and MC on spinal cord STAT3 activation. Signal transducer and activator of transcription 3 (STAT3) is a transcription factor involved in itch-related inflammatory signaling (26). Immunofluorescence staining revealed increased STAT3 activation in the spinal cord of pruritus-induced mice. In contrast, DLE and MC treatment significantly reduced STAT3 phosphorylation in both compound 48/80- and chloroquine-induced models (Fig. 5A-C). This suggests that DLE and MC exert antipruritic effects by inhibiting STAT3-mediated inflammatory signaling in the central nervous system.

Effects of DLE and MC on IL-31 expression. Interleukin-31 (IL-31) is a pruritogenic cytokine associated with chronic itch and inflammatory skin diseases (25). ELISA and Western blot analyses demonstrated elevated IL-31 levels in the serum and skin of mice injected with compound 48/80 or chloroquine. Compound 48/80 induced a more pronounced increase in IL-31 than chloroquine, consistent with its stronger histamine-dependent effects. However, pretreatment with DLE and MC significantly reduced IL-31 expression and secretion in both models (Fig. 6A-D). These results highlight the ability of DLE and MC to suppress IL-31 production, further supporting their role in alleviating pruritus.

Discussion

Pruritus, or itching, is a complex and multifaceted sensory phenomenon that significantly impacts the quality of life for affected individuals. It arises from a variety of dermatological, systemic, and neurological conditions and can be classified into histamine-dependent (6) and histamine-independent pathways (15). Histamine-dependent itching, mediated primarily by mast cell degranulation and histamine release, is often associated with acute pruritus (5). In contrast, histamine-independent itching, involving mediators such as gastrin-releasing peptide (GRP), interleukin-31 (IL-31), and signal transducer and activator of transcription 3 (STAT3), is frequently linked to chronic and refractory itch conditions (27). Understanding the mechanisms underlying these pathways is crucial for developing effective antipruritic therapies. In this study, we investigated the antipruritic effects of *D. lotus* leaf extract (DLE) and its major constituent, myricitrin (MC), in both histamine-dependent and histamine-independent itch models. Our findings suggest that DLE and MC exert significant antipruritic effects through modulation of peripheral and central itch signaling pathways.

The results of this study revealed that DLE and MC significantly reduced scratching behavior in ICR mice induced with compound 48/80 (a histamine-dependent pruritogen) and chloroquine (a histamine-independent pruritogen). These findings align with previous studies demonstrating the antipruritic properties of plant-derived polyphenols, which are known to exhibit anti-inflammatory, antioxidant, and mast cell-stabilizing effects (28-30). The reduction in scratching behavior observed in both models suggests that DLE and MC target multiple itch pathways, making them promising candidates for the treatment of diverse pruritic conditions.

Histamine is a well-established mediator of acute itching, and its release from mast cells is a hallmark of histamine-dependent pruritus (6). Our results showed that compound 48/80, a potent mast cell degranulator, significantly increased serum histamine levels and mast cell infiltration in the skin. In contrast, chloroquine, which operates through histamine-independent mechanisms, induced a milder increase in histamine levels. Treatment with DLE and MC markedly reduced serum histamine levels and mast cell infiltration in both models, indicating their ability to stabilize mast cells and inhibit histamine release. These findings are consistent with previous reports highlighting the mast cell-stabilizing effects of polyphenolic compounds, which are abundant in DLE (31-33). For instance, myricitrin has been shown to inhibit mast cell degranulation by suppressing calcium influx and downstream signaling pathways (34).

In addition to their peripheral effects, DLE and MC demonstrated significant modulation of central itch signaling pathways. GRP, a neuropeptide expressed in primary itch afferents, plays a critical role in transmitting itch signals to the spinal cord (27). Our immunofluorescence staining results revealed elevated GRP expression in the spinal cord of pruritus-induced mice, which was significantly attenuated by DLE and MC treatment. This suggests that DLE and MC may inhibit the transmission of itch signals at the spinal level, providing a potential mechanism for their antipruritic effects.

IL-31 and its receptor, IL-31RA, are key mediators of chronic itch and neuroimmune interactions. Elevated IL-31 levels have been reported in various pruritic conditions, including atopic dermatitis and chronic idiopathic pruritus (35). In this study, both compound 48/80 and chloroquine increased IL-31 expression in the serum and skin, with compound 48/80 inducing a more pronounced effect. However, treatment with DLE and MC significantly reduced IL-31 levels, suggesting that these compounds may alleviate pruritus by suppressing IL-31-mediated signaling. This effect may be linked to previous studies demonstrating that DLE and MC suppress IL-31 by inhibiting the NF-κB, MAPK, and JAK/STAT pathways at the cellular level (36). Moreover, the observed downregulation of IL-31RA expression in the spinal cord following DLE and MC treatment underscores their potential to modulate central itch pathways.

STAT3, a transcription factor involved in inflammatory signaling, has been implicated in the pathogenesis of chronic itch. Activation of STAT3 in the spinal cord contributes to the amplification of itch signals and the development of chronic pruritus (37). Notably, persistent STAT3 activation has been shown to upregulate LCN2 expression, which, in turn, exacerbates itch through the GRP/GRPR signaling pathway (26). Our results demonstrated increased STAT3 activation in the spinal cord of pruritus-induced mice, which was significantly reduced by DLE and MC treatment. This effect may be linked to previous findings demonstrating that DLE and MC suppress LCN2 expression by inhibiting IP3R1 and STAT3 activation in astrocytes (22). Furthermore, our results align with previous reports indicating that polyphenolic compounds effectively inhibit STAT3 phosphorylation and downstream inflammatory signaling (38,39).

The findings of this study have important implications for the development of novel antipruritic therapies. The ability of DLE and MC to target both peripheral and central itch pathways suggests their potential efficacy in alleviating pruritus. Their mast cell-stabilizing, histamine-lowering, and cytokine-modulating properties position them as potential candidates for the treatment of diverse pruritic conditions, including atopic dermatitis, chronic idiopathic pruritus, and drug-induced itching. Moreover, the natural origin of DLE and its constituents, such as myricitrin, offers a safer and potentially more tolerable alternative to synthetic antipruritic agents, which are often associated with adverse effects.

One of the strengths of this study is the comprehensive evaluation of both peripheral and central mechanisms underlying the antipruritic effects of DLE and MC. By employing histamine-dependent and histamine-independent itch models,

we were able to demonstrate the broad-spectrum efficacy of these compounds. This study focused on MC, the most abundant polyphenol in DLE, present at concentrations 4-100 times higher than other polyphenolic compounds. Although other polyphenols in DLE may also contribute to the anti-itch effect, MC is likely the primary active ingredient due to its predominant concentration. Exploring the synergistic effects of the other polyphenolic compounds in DLE and their combined actions on itch will provide a deeper understanding of the full spectrum of DLE's therapeutic properties. However, this study has some limitations. First, the experiments were conducted exclusively in animal models, and further research is needed to validate these findings in human subjects. Second, while we identified myricitrin as a major active constituent of DLE, the potential contributions of other polyphenolic compounds to the observed effects cannot be ruled out. Additionally, the lack of dose-response studies and long-term toxicity studies limits our ability to optimize the dosages of DLE and MC. Future studies should focus on these evaluations to establish the safety profile of DLE and MC for potential therapeutic use.

In conclusion, this study demonstrates that *D. lotus* leaf extract (DLE) and its major constituent, myricitrin (MC), exert potent antipruritic effects through multiple mechanisms, including modulation of mast cell activity, histamine release, and central itch signaling pathways. Their ability to target both histamine-dependent and histamine-independent pruritus makes them promising candidates for the development of novel antipruritic therapies. Further research is warranted to explore their efficacy in human clinical trials and to elucidate the precise molecular mechanisms underlying their antipruritic effects.

Acknowledgements

Not applicable.

Funding

This work was supported by Wonkwang University (grant no. Wonkwang University-2024).

Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JS and BK made major contributions to the study design and manuscript writing, and made substantial contributions to the acquisition, analysis and interpretation of the data. JS drafted the manuscript. SJ contributed to the study conceptualization, data curation, manuscript review and editing. JS and SJ confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by Jeonju University Institutional Animal Care and Use Committee (approval no. jj IACUC-20230602-2022-0402-C1).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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