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Suppression of skin lesions and SLE nephritis by increasing Treg in MRL/FAS^{lpr} mice by administration of bee venom Apitoxin®

Duk-Yeon Cho[1], Young-Mo Kang[2] and SangHo Seol[1*]

Abstract

Background Apitoxin®, a drug based on bee venom was approved and released in Korea in 2003 as the ethical drug (ETC). It is well-known for its pain-relieving properties due to its potent anti-inflammatory effects. This raises the question of whether bee venom has benefits for other inflammatory disorders. Since its effectiveness in treating inflammation and pain associated with autoimmune diseases has been observed in several clinical cases in Korea, we conducted an efficacy study using an animal model of the systemic lupus erythematosus (SLE), an autoimmune disease with high medical unmet needs. In this research, we aim to confirm the potential therapeutic efficacy for SLE through the immunomodulation induced by bee venom.

Methods MRL/FAS^{lpr} mice were injected subcutaneously with Apitoxin® and evaluated for clinical parameters including proteinuria, skin lesions, and lymphadenopathy, flow cytometric evaluation of regulatory T cells (Treg), quantitative evaluation of anti-dsDNA antibody in serum by ELISA, and histomorphometric analysis of kidney tissues.

Results Treatment with Apitoxin® revealed a reduction in proteinuria, skin lesions, and lymphadenopathy in MRL/FAS^{lpr} mice. The percentage of $CD3^+CD4^+CD25^+$FoxP3 (Treg) cells, which are associated with autoimmune diseases, was increased compared to the negative control (vehicle). Quantitative analysis of autoantibodies in the blood of MRL/FAS^{lpr} mice showed a decreasing tendency in the treatment groups with Apitoxin®. Moreover, mesangial proliferation and inflammatory cell infiltration in glomeruli were significantly reduced in the treatment group with Apitoxin®, which was associated with a statistically significant decrease in the amount of IgG infiltrated into the glomeruli.

Conclusion Overall, the results confirmed that Apitoxin® induced clinical improvement in SLE by increasing the proportion of Treg cells and decreasing anti-dsDNA antibodies in the blood, which resulted in therapeutic effects on glomerulonephritis associated with decreased renal infiltration of immune complexes.

Keywords Bee venom, Apitox, Apitoxin, Systemic lupus erythematosus, MRL/FAS^{lpr}, Regulatory T cell

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Introduction

Systemic lupus erythematosus (SLE) is a multifactorial disease with genetic predisposition, environmental factors, and perturbations in both innate and adaptive immunity, including defective apoptotic cell clearance, cytokine imbalances, B-cell hyperreactivity, and disturbances in T-cell signal transmission [1–3]. The pathogenesis is characterized by autoantibodies against cellular and nuclear components, and antibody-mediated histopathologic findings are observed in the skin, joints, kidneys, heart, central nervous system, and lungs. This results in a variety of symptoms and, in severe cases, can lead to organ failure or death [4, 5].

Although new drugs targeting various aspects of the pathogenesis are being developed, the number of agents approved for use remains very limited, making it one of the diseases with the highest unmet medical needs [6, 7]. Animal models are essential for better understanding the pathogenesis of SLE, which results from complex interactions among various cells. The importance of animal models has been increasingly emphasized in recent years, especially in translational research to identify the efficacy and toxicity of new agents prior to human clinical trials. The MRL/FAS^{lpr} mouse, which has a null allele for Fas (CD95), has a defect in the cell death pathways of the immune system, resulting in the accumulation of a large and distinct population of B220^{+} B cells and CD4^{neg}CD-8^{neg} (double negative, DN) T cells. This mouse model is the most commonly used model for pathogenesis studies and preclinical studies for drug discovery, as it exhibits features very similar to human lupus, such as skin lesions, nephritis, lymphadenopathy, and autoantibody production [8].

Bee venom *(Apis mellifera* L,.) is a natural toxin that has long been used as a traditional medicine in Egypt and the East [9–12]. Apitoxin® was a synonym for dried bee venom, and is the name of the drug administered via intradermal injection that was approved by the Korean Food and Drug Administration (KFDA) in South Korea, in 2003. Bee venom is a complex compound composed of several substances reported to have beneficial effects on cancer, arthritis, liver disease, diabetes, and more. Key components such as melittin and phospholipase A2 (PLA2) are known to play crucial roles [12]. The main component of the drug is melittin, comprising 52% of venom peptides, while PLA2 makes up 11%. Melittin in bee venom has been published to possess anti-cancer [13–15], anti-inflammatory [16–18], anti-arthritic [19–21], and antibacterial properties [22]. PLA2, the second most abundant peptide in bee venom, plays an important role in regulating the immune response by increasing the expression of FOXP3, a transcription factor of regulatory T cells (Treg), and is thus expected to have a significant role in autoimmune diseases [23–25].

It has been reported that bee venom reduces inflammation by inhibiting the secretion of proinflammatory cytokines such as Tumor Necrosis Factor-α (TNF-α), Interleukin (IL)-1β, and IL-6. Additionally, components of bee venom play a critical role in modulating the inflammatory response by blocking the nuclear factor kappa-light-chain-enhancer of activated B cell (NF-κB) pathway, which regulates the expression of inflammation-related genes. Furthermore, the suppression of inflammation-related enzymes, including cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), is recognized as one of the anti-inflammatory mechanisms of bee venom [26, 27]. Bee venom exhibits multiple mechanisms that allow it to act as an immunomodulator in autoimmune inflammatory diseases. Specifically, bee venom promotes immune balance by activating Tregs and modulating the Th1/Th2 immune response to alleviate inflammation [28, 29]. Additionally, it is known to inhibit the secretion of pro-inflammatory interleukins, such as IL-17 and IL-12, and selectively induce apoptosis in overactivated immune cells responsible for driving inflammation [26]. We hypothesized that Apitoxin® may have a therapeutic effect on the SLE through its anti-inflammatory and immune regulatory effects.

This study aimed to investigate the therapeutic efficacy of Apitoxin® in an SLE animal model, the MRL/FAS^{lpr} mouse, by evaluating clinical parameters, histopathologic scores, and biomolecular markers.

Materials and methods

Mice

MRL/FAS^{lpr} mice were purchased from Japan SLC (Hamamatsu, Japan). Mice were received at 10 weeks of age, acclimatized for 1 week and started the study at 11 weeks of age. Mice were treated with control and test substances for 10 weeks from 11 weeks of age. All animal experiments were performed according to the approved animal protocols and guidelines by the IACUC of Kyungpook National University School of Medicine.

Animal experiment

Forty female MRL/FAS^{lpr} mice were housed in a specific pathogen free (SPF) facility and placed in an environmentally controlled room maintained at a temperature 23 ± 3℃, a relative humidity of 55 ± 15%, a ventilation frequency of 10–20 times/h, a lighting time of 12 h/day (8 am to 8 pm), and a illuminance of 150–300 lx. Food and water were freely available to the mice. The mice were divided into six groups for the experiment: the normal control C57BL/6 group (G1), and MRL/FAS^{lpr} mice treated with vehicle (Normal saline, G2), mycophenolate mofetil (60 mg/kg, QD, P.O., G3), and three different doses of Apitoxin®, namely lower dose (G4), middle dose (G5), and high dose schedules (G6). Apitoxin® was

administered to the mice in G4, G5 and G6 twice a week for 10 weeks via subcutaneous injection.

Therapeutic dose schedule

After weighing the 2 mg/mL of Apitoxin®, it was diluted to a 0.2 mg/mL concentration using normal saline to prepare a stock concentration. Each group received Apitoxin® in an escalating dosing schedule of every 2-week interval. G4 received 0.04 mg/kg of Apitoxin® in the 1–2 weeks, 0.08 mg/kg in the 3–4 weeks, 0.16 mg/kg in the 5–6 weeks, and 0.24 mg/kg in 7–10 weeks. G5 received 0.32 mg/kg in the 1–2 weeks, 0.64 mg/kg in the 3–4 weeks, 0.96 mg/kg in the 5–6 weeks, and 1.2 mg/kg in 7–10 weeks. G6 received 0.375 mg/kg in the 1–2 weeks, 0.75 mg/kg in the 3–4 weeks, 1.5 mg/kg in the 5–6 weeks, and 3 mg/kg in 7–10 weeks. Vehicle was administered in G1 and G2 at each injection days.

Clinical assessments

Preclinical assessment was determined by evaluating proteinuria, lymphadenopathy, skin lesions, body weight, and survival rate. From the date of group separation to the end of the experiment, the occurrence and severity of diseases were observed and evaluated regularly. Proteinuria was assessed by semi-quantitative measurement of protein concentration in mouse urine once a week, and proteinuria score were evaluated according to the evaluation criteria. A proteinuria score was assigned using a scale of 0 to 4, with 0 being negative; 1, (0.3 g/L); 2, (1 g/L); 3, (3 g/L); and 4, (≥ 20 g/L). Skin score assesses the severity of skin lesions in the periorbital, facial, and back regions based on the evaluation. A skin score was assigned using a scale of 0 to 4, with 0 being none; 1, 1 or 2 small lesions (2–4 mm) in face, minimal hair loss of redness; 2, lesion larger than 1 but total area < 5 cm^2 in face and ears, redness, scabbing and hair loss; 3, lesion with total area ≥ 5 cm^2 < 1 cm^2 face and ears and back, ulcerations with an extensive area of involvement; and 4, lesion with total area ≥ 1 cm^2 face and ears and back, ulcerations with an extensive area of involvement. A lymphadenopathy score was assigned using a scale of 0 to 3, with 0 being none; 1, small and one site; 2, moderate and two different sites; and 3, large and three different sites.

Immune cell subset analysis by flow cytometry

Cell isolation was performed using a cell strainer from mouse spleen obtained after administration oftest substances. Cells were harvested per group, washed with 0.1% BSA-PBS and treated with Fc receptor binding inhibitor (Invitrogen, 14-9161-73) for10 minutes at 4 °C. Cells were then reacted with antibodies against cell surface markers (CD3, CD4, and CD25) for 30 min at 4 °C. To proceed with staining for intracellular markers, cells were washed and incubated with fixation/permeabilization concentrate (eBioscience, 00-5123-43) for 45 min at 4 °C, followed by 1x Permeabilization Buffer at 4 °C for 15 min, and then incubated with FoxP3 antibody for 40 min at 4 °C. Finally, the cell pellet collected after washing was resuspended in 2% paraformaldehyde (Biosesang, Daegu, Korea), cells were acquired using a flow cytometer (NovoCyte with NovoSampler Pro, Agilent) and analyzed by NovoExpress software (Agilent Technologies, Inc.). The following anti-mouse antibodies were used: anti-CD3-FITC (Biolegend, 100204), anti-CD4-PerCP (Biolegend, 100538), anti-CD25-APC (Biolegend, 101910), anti-FoxP3-PE (12-5773-82).

Plasma ELISA for anti-dsDNA concentration

Plasmas obtained after treatment with the test substances were diluted 1/1,500 and incubated for 2 h with a mouse anti-dsDNA antibody (Chondrex, 3031)-coated plate that had been previously treated with blocking buffer at room temperature (RT) for 1 h. After washing with wash buffer, the secondary antibody (HRP-conjugated) was incubated for 1 h. The plate was then further washed with wash buffer, and reacted with TMB substrate solution for 25 min, after which the stop solution was added. The absorbance was then measured at 450 nm using a microplate reader, Synergy HTX Multimode Reader (BioTek, Agilent).

Histopathological scoring of hematoxylin and eosin-stained tissues

Kidney tissue was isolated, fixed in 10% neutral buffered formalin solution (BBC Biochemical, 151SMIRA01), paraffin embedded, and cut into 3 μm thick sections. The sections were then stained with hematoxylin and eosin (BBC Biochemical, 3550, 3610), followed by mounting, and examination. Stained tissue slides were scanned using a digital pathology scanner (Motic Easyscan One). Four section areas (400x magnification) of each tissue were captured, and two researchers independently evaluated the severity of glomerular lesions. A glomerular lesion score was assigned using a scale of 0 to 3, with 0 being no recognizable lesion in glomeruli; 1, mild cell proliferation and/or cell infiltration; 2, same as grade 1 with mesangial proliferation, lobulation and hyaline droplet, associated with macrophage infiltration; and 3, same as grade 2 with crescent and granuloma formation and/or hyalinosis. The average of the researchers' evaluations is called the scoring value, and the average of the four scoring values is calculated as the score for each tissue.

Histomorphometric analysis of immunofluorescence (IF) staining for immunoglobulin G (IgG)

Kidney tissue was isolated, OCT-embedded, and cut 7 μm thick sections. Tissue slides were stained with Alexa

Fluor 488-conjugated goat anti-mouse IgG antibody (Abcam, ab150117), Nuclear staining was performed using DAPI (Thermo, 62248, 1 μL/mL in PBS), and tissue slides were mounted with anti-fade mounting media (Sigma Aldrich, 03989). The stained tissue slides were photographed using a laser scanning confocal microscope (Nikon $C2^+$) at four areas (400x magnification) for each tissue. The fluorescence intensity of IgG-stained glomerular lesions was measured using Image/J software (NIH, Bethesda, MD), utilizing the same circle for all sections. The fluorescence intensity was obtained by averaging the measurements of the 4 foci.

Statistical analysis

Statistical analyses between the negative control group and the test group, or between the two test groups, based on blinded assessment data for each endpoint, were performed using SPSS (IBM Corp.v26.0). Independent *t* test was used for comparisons between groups. For comparison of repeated trials, repeated measures ANOVA with Tukey's post-hoc test was used. Linear correlations between evaluation indicators were analyzed using Spearman's correlation coefficients. A *P* value of 0.05 or less was considered significant.

Results

Apitoxin® improved symptoms of SLE in MRL/FAS^{lpr} mice

To evaluate the safety of Apitoxin®, body weights and survival rates were measured weekly from the start of treatment until the end of the study. The safety evaluation showed that one mouse died at week 8 in the negative control group, and no animals died in all other treatment groups, confirming that Apitoxin® had at least no adverse effects on survival. In addition, there were no statistically significant body weight changes in the MRL/FAS^{lpr} mice treated with either the negative control group or different dose groups of Apitoxin® treatment (Supplementary Fig. 1). To investigate the clinical improvements, vehicle, mycophenolate mofetil, and Apitoxin® were administered weekly for 10 weeks starting at 11 weeks of age. Analysis of clinical parameters showed that Apitoxin® treatment resulted in improvement in the development and severity of lupus (Figs. 1 and 2). Proteinuria scores showed a decrease in the positive control group and all Apitoxin® treatment groups compared to the negative control group (Fig. 2A). Analysis of the area under the curve (AUC) of the proteinuria scores showed a statistically significant decrease in the positive control group and the middle and high dose groups of Apitoxin® treatment compared to the negative control group (Fie. 2B). Incidence of moderate to severe proteinuria (Proteinuria score ≥ 2) was found to be reduced in all Apitoxin® treatment groups except for the Apitoxin® dose B group compared to the negative control group (Supplementary Fig. 2). Taken together, these results showed the therapeutic effects of Apitoxin® on the severity of nephritis.

Skin scores showed a clear progression over time in the negative control group and a dose-dependent reduction in three different dose groups of Apitoxin® treatment (Fig. 2C). Skin score AUC values showed a statistically significant decrease in the positive and high dose Apitoxin® groups compared to the negative control group, with a dose-dependent decrease among the three different dose groups of Apitoxin® (Fig. 2D). Lymphadenopathy scores and score AUC values showed a decrease in all Apitoxin® treatment groups compared to the negative control, with a statistically significant decrease in the middle and high doses groups of Apitoxin® treatment compared to the negative control group. (Fig. 2E and F). These results implicate that Apitoxin® had a therapeutic effect on skin rashes and lymphadenopathy, especially in the high dose group. Kidney weights measured in MRL/FAS^{lpr} mice (Supplementary Fig. 3A-C), were similar between the negative control group and the positive and Apitoxin® groups.

Apitoxin® increased Treg cells from MRL/FAS^{lpr} mice

Although the causes of many autoimmune diseases, including lupus, remain largely unknown and are believed to be multifactorial, impaired function of Treg cells may play an important role in the development of autoimmune diseases [30–33]. Therefore, we analyzed changes in Treg cells using spleens from the MRL/FAS^{lpr} mouse model to determine the regulation of immune processes in response to treatment. The proportion of $CD3^+CD4^+CD25^+FoxP3^+$ (Treg) cells among $CD4^+$ T cells increased in all Apitoxin® treatment groups compared to the negative control group, with a statistically significant increase in the middle and high doses groups of Apitoxin® treatment (Fig. 3).

Apitoxin® reduced anti-dsDNA antibody production

SLE is an autoimmune disease characterized by a variety of autoantibody productions. The detection of anti-double-stranded DNA (dsDNA) antibody is crucial for the diagnosis of SLE [34] and is included in the American College of Rheumatology classification criteria for SLE [35]. Moreover, the correlation of anti-dsDNA antibody levels with lupus nephritis activity has been reported, making quantitative testing of anti-dsDNA antibody useful for pre- and post-treatment follow-up of SLE [36–38]. Therefore, we quantitatively analyzed anti-dsDNA, IgG concentration in the blood of MRL/FAS^{lpr} mice. The concentration of anti-dsDNA antibody showed a decreasing tendency in all Apitoxin® treatment groups compared to the negative control group, although statistical significance was not demonstrated (Supplementary Fig. 4). Our study demonstrated an increase in Treg cells following

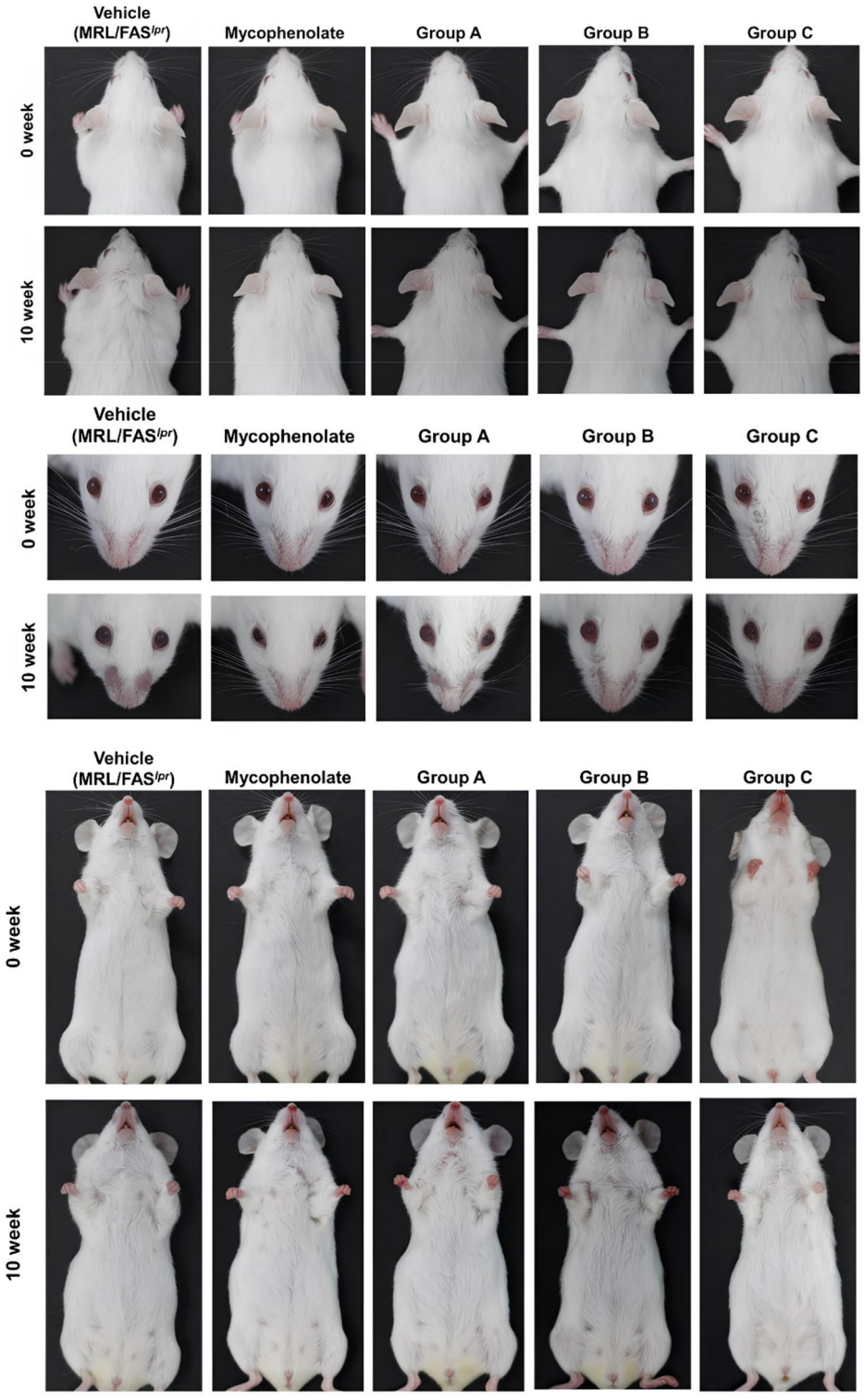

Fig. 1 Representative gross pictures of skin lesions and lymphadenopathy in MRL/FAS^{lpr} mice following treatment of indicated compounds for 10 weeks. The mice were grouped into C57BL/6 and MRL/FAS^{lpr} mice treated with Vehicle, Mycophenolate (PO, QD), A (SC, TIW), B (SC, TIW), and C (SC, TIW). G2, Vehicle-MRL/FAS^{lpr}; G3, Mycophenolate; G4, low dose of bee venom; G5, middle dose of bee venom; G6, high dose of bee venom

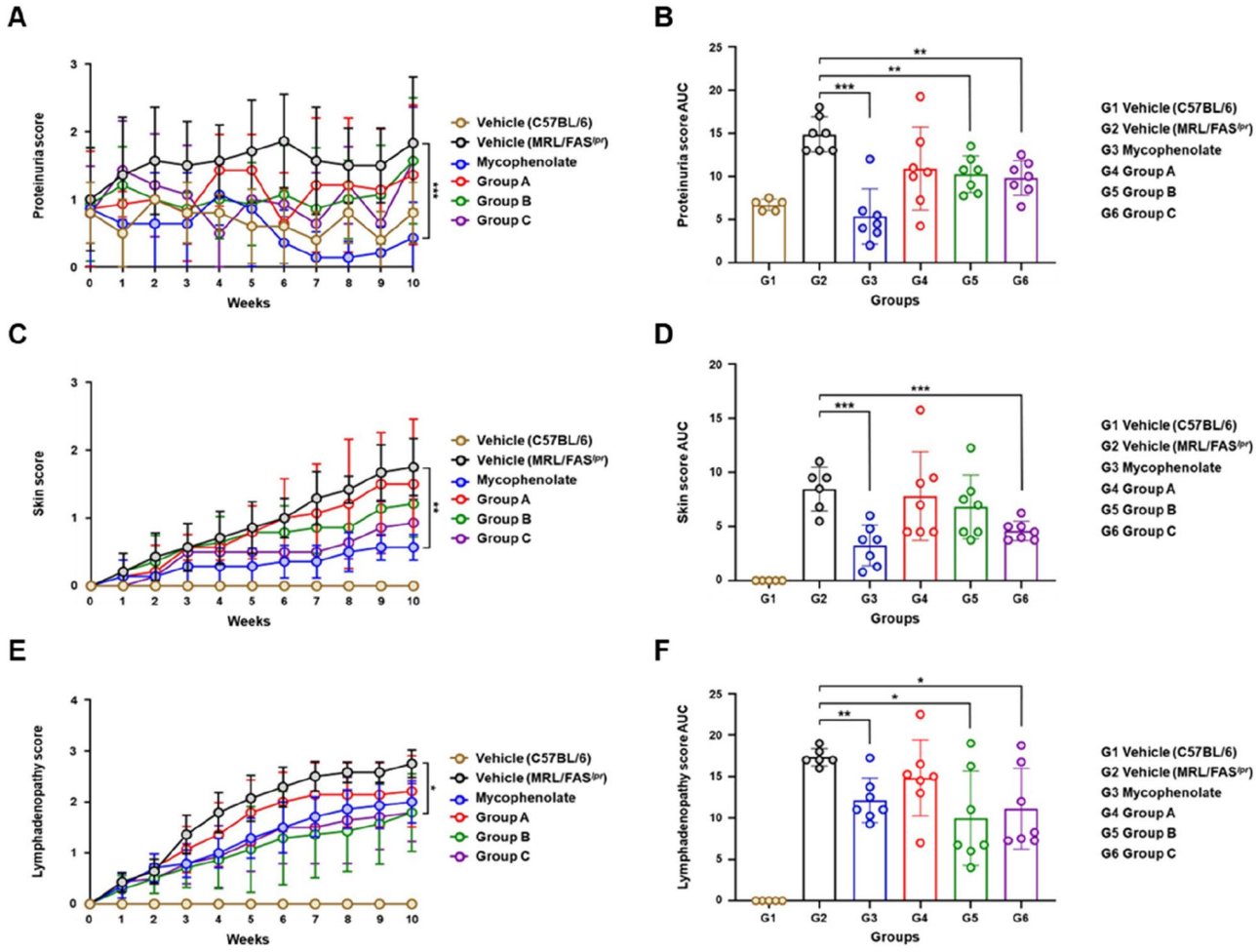

Fig. 2 Proteinuria score or Area under the curve (AUC) (**A**-**B**), Skin scores or AUC (**C**-**D**), and Lymphadenopathy scores or AUC (**E**-**F**) of Normal and MRL/FAS^{lpr} mice following continuous treatment of indicated compounds for 10 weeks. The mice were grouped into C57BL/6 and MRL/FAS^{lpr} mice treated with Vehicle, Mycophenolate (PO, QD), A (SC, TIW), B (SC, TIW), and C (SC, TIW). Data are expressed as the mean ± SD. * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ *versus* Vehicle (MRL/FAS^{lpr}). G1, C57BL6; G2, Vehicle-MRL/FAS^{lpr}; G3, Mycophenolate; G4, low dose of bee venom; G5, middle dose of bee venom; G6, high dose of bee venom

Apitoxin® administration. Treg cells play a crucial role in maintaining immune tolerance by suppressing autoreactive B cell activation and autoantibody production. Previous studies have reported that impaired Treg function contributes to elevated autoantibody levels in SLE, and enhancing Treg activity can mitigate disease progression [39, 40]. Thus, the observed decrease in anti-dsDNA antibody levels, although not statistically significant, may be due to the suppression of B cell hyperactivity mediated by the increased Treg population.

Apitoxin® ameliorated renal infiltration of immune complexes

During the course of glomerulonephritis in this model, inflammatory immune cells such as macrophages and neutrophils infiltrate the kidney. In the negative control group, a large number of inflammatory cell infiltrations within glomeruli, along with mesangial proliferation, renal tubule destruction, and inflammatory cell infiltration in the tubulointerstitial space, were observed. In some of the glomeruli, crescent formation by epithelial proliferation was also interspersed. However, in the Apitoxin® treatment groups, both mesangial proliferation and inflammatory cell infiltration within the glomeruli were significantly reduced (Fig. 4). Based on this, the glomerulonephritis score was reduced in all Apitoxin® treatment groups compared to the negative control group, with a statistically significant reduction in the middle and high dose groups of Apitoxin® treatment (Supplementary Fig. 5).

IgG antibodies are a key component of adaptive humoral immunity, but they can cause organ damage if they bind self-antigens, as occurs in the autoimmune disease SLE. In this study, immunofluorescence staining of kidney tissues for IgG revealed that the amount of IgG infiltrated into the glomeruli was significantly increased

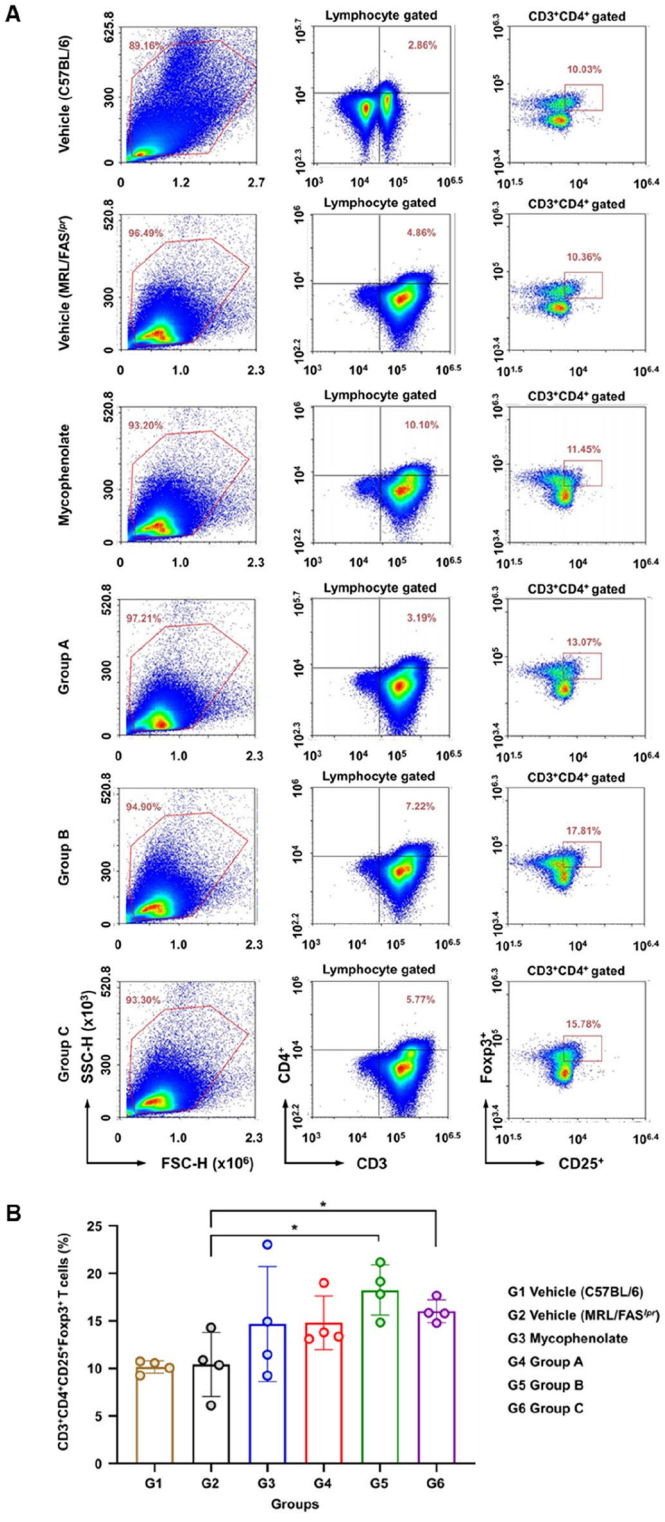

Fig. 3 Representative figures of regulatory T (Treg) cell subsets on flow cytometry (FOXP3 as a marker for Treg cells) in the spleen of Normal and MRL/FAS^{lpr} mice following treatment of indicated compounds for 10 weeks. The mice were grouped into C57BL/6 and MRL/FAS^{lpr} mice treated with Vehicle, Mycophenolate (PO, QD), A (SC, TIW), B (SC, TIW), and C (SC, TIW). After staining, cells were first gated for $CD3^+CD4^+$ cells, and the $CD25^+FOXP3^+$ cells were analyzed by flow cytometry. G1, C57BL6; G2, Vehicle-MRL/FAS^{lpr}; G3, Mycophenolate; G4, low dose of bee venom; G5, middle dose of bee venom; G6, high dose of bee venom

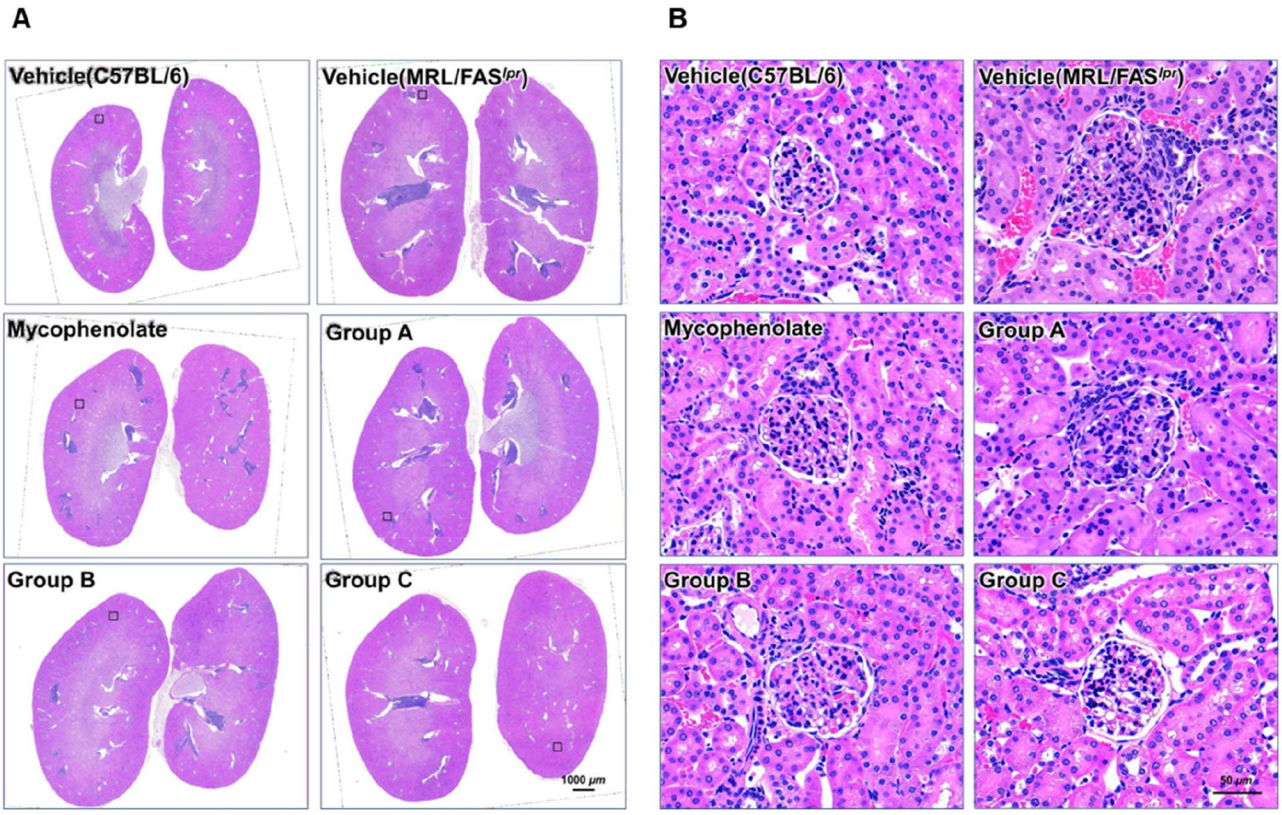

Fig. 4 Representative pictures of H&E-stained kidney tissues from Normal and MRL/FAS^{lpr} mice following treatment of indicated compounds for 10 weeks. The mice were grouped into C57BL/6 and MRL/FAS^{lpr} mice treated with Vehicle, Mycophenolate (PO, QD), A (SC, TIW), B (SC, TIW), and C (SC, TIW). H&E stain; Magnification x 9.2; Scale bar 1000 μm (**A**) and x 400; Scale bar 50 μm (**B**). G1, C57BL6; G2, Vehicle-MRL/FAS^{lpr}; G3, Mycophenolate; G4, low dose of bee venom; G5, middle dose of bee venom; G6, high dose of bee venom

in the negative control group, while it is decreased in all Apitoxin® treatment groups (Fig. 5A). The fluorescence intensity of IgG infiltration was measured and showed a statistically significant reduction in all Apitoxin® treatment groups compared to the negative control (Fig. 5B). Taken together, these results showed that Apitoxin® has a therapeutic effect on glomerulonephritis through the reduction of renal infiltration of immune complexes.

Discussion

Bee venom, in its raw form, is associated with a high risk of hypersensitivity reactions, including anaphylactic shock, due to the presence of various impurities [41, 42]. Purification processes remove these impurities and concentrate bioactive components like melittin, enhancing safety and therapeutic efficacy [12, 43]. Apitoxin®, a highly purified bee venom product, has demonstrated significant efficacy in alleviating osteoarthritis pain [44, 45]. Several studies have demonstrated that Apitoxin® effectively reduces pain scores and inflammatory cytokine levels in mouse models of arthritis and neuropathic pain in rats [46, 47]. Clinical trials have corroborated these findings, indicating the same efficacy in human subjects [45]. Given Apitoxin®'s well-established anti-inflammatory properties, it has been explored for various inflammatory conditions, including autoimmune diseases [48]. However, its efficacy in lupus has been studied less extensively, warranting further investigation.

In a model of the lupus nephritis, the study results demonstrated that treatment with Apitoxin®, significantly alleviated symptoms of skin rash and nephritis. The extent of symptom relief observed following Apitoxin® treatment surpassed initial expectations. The results indicated that the Apitoxin® treatment groups showed reductions in proteinuria, skin lesions, and lymphadenopathy. Importantly, a dose-dependent reduction in glomerulonephritis severity, comparable to that observed in the positive control group, was noted in the Apitoxin® treated group.

Lupus is characterized by the production of antibodies against nuclear antigens, leading to the formation of immune complexes that become deposited, particularly in the glomerulus, causing glomerulonephritis [49–52] Based on this understanding, we confirmed through immunostaining that IgG deposition decreased in the Apitoxin® treatment group. This result indicates a reduction in immune complexes, which play a key role in lupus nephritis by triggering complement activation and

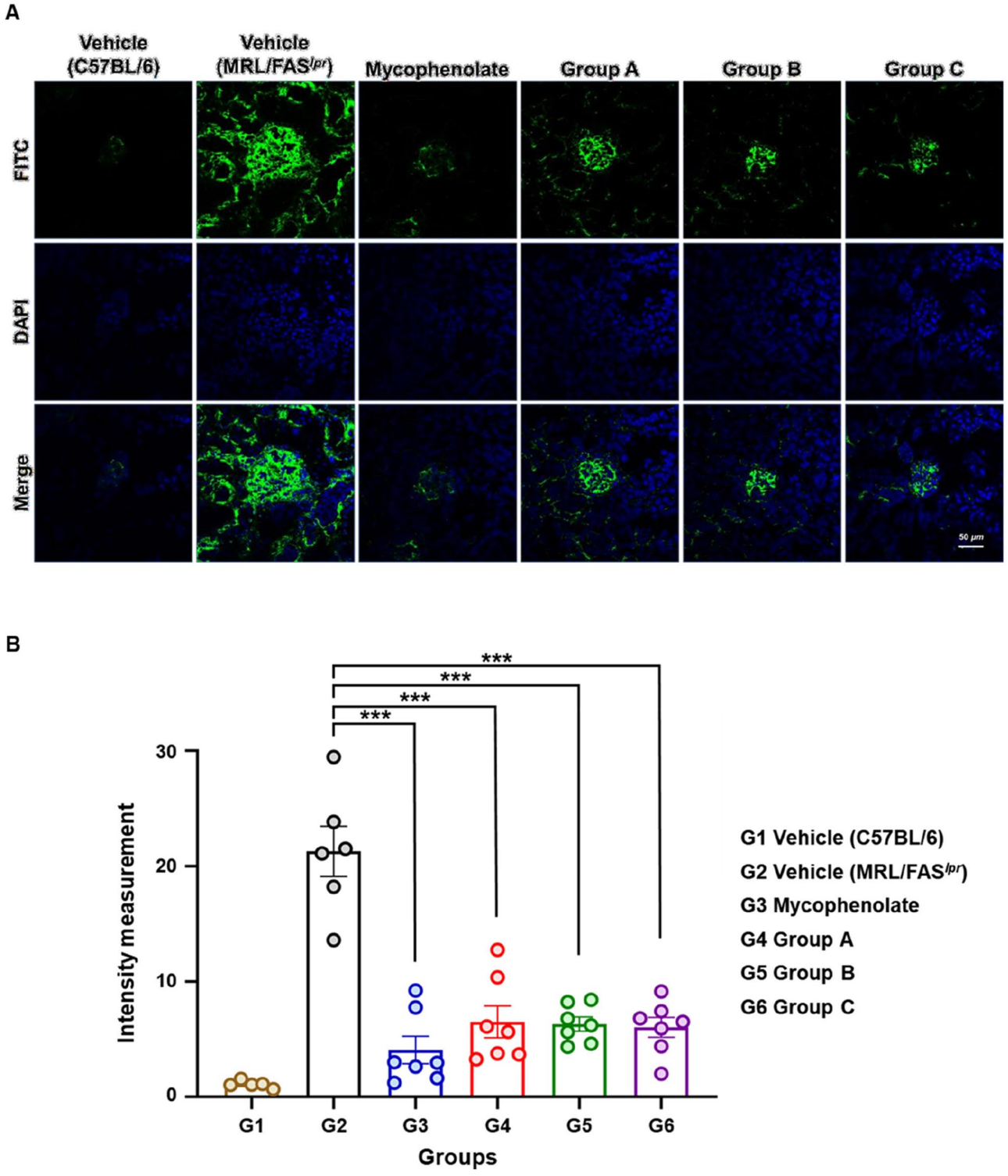

Fig. 5 Representative figures of IgG-stained kidney tissues from Normal and MRL/FAS^{lpr} mice following treatment of indicated compounds for 10 weeks. The mice were grouped into Normal (C57BL/6) and MRL/FAS^{lpr} mice treated with Vehicle, Mycophenolate, Group A, Group B, Group C. IgG immunofluorescence stain, Magnification x 400, Scale bar 50 *μm*. G1, C57BL6; G2, Vehicle-MRL/FAS^{lpr}; G3, Mycophenolate; G4, low dose of bee venom; G5, middle dose of bee venom; G6, high dose of bee venom

inflammatory responses [53, 54]. Previous studies have shown that therapeutic agents reducing immune complex deposition, such as belimumab, can improve renal function in lupus [55]. Therefore, the observed reduction in IgG deposition suggests a potential improvement in glomerulonephritis, though further investigation is needed.

In general, among the multiple elements in SLE pathogenesis, B cells play a central role in SLE through both antibody-dependent and antibody-independent manners [56]. Autoantibody levels are significantly increased by B cell hyperactivity, and this series of responses is regulated by Treg cells. Suárez-Fueyo et al. demonstrated that the proportion or function of a subset of T cells is abnormal in SLE patients. When Treg cell function is impaired, autoimmune diseases can develop, and in some cases, Treg cells fail to do their job and become inflammatory [57]. Therefore, there are various attempts to suppress the inflammatory response in autoimmune diseases by enhancing Treg cell function [58]. In this study, the percentage of $CD3^+CD4^+CD25^+FoxP3$ (Treg) cells was increased in the Apitoxin® treated group compared with the negative control group. Although the exact mechanism is not elucidated in this study, it appears that Apitoxin® treatment primarily decreases B cell hyperactivity by increasing Treg cells, which in turn results in the reduction of autoantibody production. Although statistical significance was not demonstrated, this reduction might be attributable to possible mechanisms, such as the immunomodulatory effects of the Apitoxin®, which could suppress autoantibody production or promote immune tolerance. Similar findings have been reported in previous studies, where some therapeutic agents (e.g., belimumab, anifrolumab) did not show statistically significant reductions in anti-dsDNA levels in lupus animal models, while demonstrating clinical efficacy in human trials [59, 60]. Further studies are needed to clarify the exact mechanism underlying this effect.

Although further studies on the direct therapeutic mechanisms of action and administration methods that can enhance the efficacy of Apitoxin® are needed in the future, this study showed that Apitoxin® can effectively treat lupus and lupus nephritis in an MRL/*lpr* animal model by increasing Treg cells and thereby decreasing antibody production, as evidenced by reduced skin lesions, proteinuria, lymphadenopathy, and anti-dsDNA antibodies. It also demonstrated effectiveness in treating lupus nephritis by Treg cells, reducing mesangial proliferation in kidney tissue, decreasing inflammatory cell infiltration in glomeruli, and decreasing the amount of IgG infiltrated into glomeruli.

While our study demonstrates the therapeutic potential of Apitoxin® in lupus and lupus nephritis through the modulation of Treg cells and reduction of IgG deposition, further research is warranted to elucidate its precise mechanisms of action. First, additional studies are needed to determine how Apitoxin® specifically regulates Treg cells and suppresses B cell hyperactivity at the molecular level. Investigating key signaling pathways involved in Treg cell expansion and function, such as the FOXP3 and IL-10 pathways, may provide deeper insights into its immunomodulatory effects. Lastly, given that our study was conducted in an MRL/lpr mouse model, further validation in other lupus models and ultimately in clinical trials is necessary to assess the translational potential of Apitoxin® in human SLE treatment. Additionally, evaluating its long-term safety profile, particularly regarding hypersensitivity reactions, will be critical for future clinical applications.

Conclusion

In conclusion, our study showed that Apitoxin® can effectively treat lupus and lupus nephritis in MRL/*lpr* animal models by alleviating disease activity, as evidenced by the reduction of skin lesions, proteinuria, lymphadenopathy and the concentration of anti-dsDNA antibody. It also demonstrated effectiveness in treating lupus nephritis by increasing Treg cells, which suppress mesangial proliferation in kidney tissues, reduce inflammatory cell infiltration in glomeruli, and downregulate the amount of IgG infiltrated into glomeruli. Further experiments are needed to elucidate its mechanisms in various immune cells and tissues to clarify the exact mechanism.

Abbreviations

ANAs	Anti-nuclear antibodies
COX-2	Cyclooxyganase-2
dsDNA	Double-stranded DNA
IF	Immunofluorescence
IgG	Immunoglobulin G
iNOS	Inducible nitric oxide synthase
IL	Interleukin
KFDA	Korean Food and Drug Administration
LN	Lymph node
NET	Neutrophil extracellular trap
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cell
PLA2	Phospholipase A2
SLE	Systemic Lupus Erythematosus
TNF-α	Tumor necrosis factor-α
Treg	Regulatory T cells

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42358-025-00448-5.

Supplementary Material 1

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Not applicable.

Author contributions

DY did conceptualization, formal analysis, data curation and writing-original draft. YM did methodology, visualization and writing-original draft. SH did project administration and supervisions. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Animal experiments were approved and performed in accordance with the approved guidelines of the Institutional Animal Care and Use Committee of Kyungpook National University, Republic of Korea (2024 – 0229).

Consent for publication
Not applicable.

Clinical trial number
Not applicable.

Competing interests
The authors declare no competing interests.

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