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ORIGINAL PAPER

Yaling Xu, Han Li, **Dioscin attenuates lupus nephritis in NZB/W F1 mice**

Dioscin attenuates lupus nephritis in NZB/W F1 mice by decreasing NF-κB activation and NLRP3 inflammasome

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Abstract

Introduction. Dioscin, a natural steroid saponin, has anticancer, anti-inflammatory, anti-hyperlipidemic, and glycemic capabilities. This study focused on dioscin roles and its related mechanisms in experimental lupus nephritis.

Materials and methods. Lupus-prone NZB/W F1 mice were intragastrically administered with dioscin, prednisone or vehicle, and kidney, urine and blood samples were harvested after the mice were sacrificed. Proteinuria, blood urea nitrogen (BUN), creatinine, anti-dsDNA, IL-1β, and IL-18 levels in serum as well as IFN-γ, IL-6, IL-17 and TNF-α levels in kidney tissues were assessed. Renal histopathology was examined through hematoxylin-eosin staining. IgG and C3 expression in kidney was evaluated using immunofluorescence staining. The number of glomerular F4/80-positive cells and NLRP3-positive cells was determined by immunohistochemical staining. The protein expression was examined by western blotting.

Results. Dioscin alleviated lupus nephritis in NZB/W F1 mice. Dioscin declined serum anti-

dsDNA level, prevented deposition of immune complexes in renal glomeruli, and inhibited the inflammatory response and infiltration of macrophages into mouse kidneys. Dioscin inhibited NF-κB and NLRP3 inflammasome in NZB/W F1 mice.

Conclusions. Dioscin ameliorates lupus nephritis through inhibition of NLRP3 inflammasome and NF-κB signaling.

Keywords: NZB/W mice; lupus nephritis; dioscin; immune complexes; NF-κB; NLRP3 inflammasome

INTRODUCTION

Systemic lupus erythematosus (SLE) refers to an autoimmune disorder involving one or more organs. Epidemiological research suggests that 0.4 million individuals are newly diagnosed with SLE worldwide, and the yearly incidence of SLE is 5.14 per 100,000 people [1]. Lupus nephritis, a frequent and severe manifestations of SLE, occurs in approximately 40% of SLE patients within 5 years, and approximately 4.3–10.1% of patients with lupus nephritis develop end-stage renal disease [2]. Renal dysfunction with inflammatory response and glomerulonephritis resulting in proteinuria is the major characteristics of lupus nephritis [3]. IgG, IgA, IgM, C1q and C3 are recognized as pathological biomarkers of lupus nephritis [4]. In human lupus nephritis, substantial infiltration of inflammatory cells, mainly macrophages, is observed in kidneys [5]. It has been reported that anti-dsDNA antibodies have diagnostic and prognostic values in lupus nephritis [6]. Currently, corticosteroids and cyclophosphamide are widely used for lupus nephritis treatment. Despite therapeutic improvements, lupus nephritis can increase mortality risk in SLE patients [7]. Thus, exploring the pathomechanisms associated with lupus nephritis and developing novel therapies for lupus nephritis treatment are urgently needed.

The NLRP3 inflammasome consists of NLRP3, ASC protein and pro-cascapse-1 [8]. Patients with lupus nephritis exhibit higher levels of NLRP3 inflammasome, IL-1β, and IL-18 in the kidney than healthy controls [9, 10]. In several lupus nephritis mouse models, the

NLRP3 inflammasome and its components are upregulated in the kidney [11, 12]. In lupus nephritis, activation of NLRP3 inflammasome in podocytes can lead to severe proteinuria [13, 14]. Inactivation of NLRP3 inflammasome has been shown to ameliorate severe lupus nephritis in mice [13, 15, 16].

NF-κB, transcription factor, is a critical inflammation regulator [17]. Under steady state, NF-κB is sequestered by IκB in cytosol. Upon activation, the kinase complex phosphorylates and degrades IκB, inducing nuclear translocation of NF-κB [18]. Activated NF-κB and elevated inflammatory cytokines were observed in glomeruli of lupus nephritis patients, and the lupus nephritis severity is associated with NF-κB activation [19]. NF-κB inactivation can ameliorate lupus nephritis pathogenesis [20, 21].

Dioscin, a natural steroid saponin, is an active ingredient in Dioscoreaceae herbs, and its anti-carcinogenic, anti-inflammatory, anti-hyperlipidemic and glycemic properties have been well-established [22]. The renoprotective effects of dioscin have been previously documented. For example, dioscin suppresses inflammation and renal ischemia/reperfusion injury *via* inactivating TLR4/MyD88 signaling [23]. Additionally, dioscin exerts protective effects against renal fibrosis by inhibiting the inflammatory response mediated by NF-κB signaling [24]. Moreover, dioscin alleviates diabetic nephropathy in mice by blocking TLR4/NF-κB signaling [25]. Furthermore, dioscin has been found to alleviate silica-aggravated SLE through inhibiting apoptosis and improving LC3-associated phagocytosis in MPL/lpr mice [26]. The effect of dioscin on inactivating NLRP3 inflammasome in periodontitis has also been reported previously [27]. Given the above, we speculated that dioscin might alleviate lupus nephritis.

Consequently, this study focused on dioscin roles and its related mechanisms in lupus nephritis. We hypothesized that dioscin might attenuate lupus nephritis pathomechanisms. This study provides evidence for the therapeutic potential of dioscin in the treatment of lupus nephritis.

MATERIALS AND METHODS

Animals

Female NZB/W F1 mice (21 weeks of age; Slack Laboratory, Shanghai, China) and wild-type C57BL/6 mice (Charles River Laboratories, Beijing, China) were housed in a specific pathogen-free animal research facility at 25 ± 2℃ with 65% of humidity under a 12-h light/dark cycle. The mice were divided into 4 groups, namely control group, NZB/W F1 group, NZB/W F1 + Dioscin group, and NZB/W F1 + Prednisone group. Each group had 10 mice. Dioscin (60 mg/kg; purity 99.76%, MedChemExpress, Shanghai, China) and prednisone (purity 99.84%; MedChemExpress) were dissolved in 0.5% sodium carboxymethyl cellulose (CMC-Na; Sigma-Aldrich, Shanghai, China) solution in distilled water. Mice were intragastrically administered with dioscin (60 mg/kg), prednisone (5 mg/kg) or vehicle (0.5% CMC-Na) from 23 weeks of age (3 times /week). The mice were sacrificed under isoflurane anesthesia at 30 weeks of age (Fig 1A). Urine and blood samples were collected followed by collection of kidney tissues. The dioscin and prednisone doses were determined according to previous publications [25, 28]. Laboratory procedures were approved by Ethics Committee of Wuhan Sixth Hospital. All the animal care was performed according to the Guide for Care and Use of Laboratory Animals.

Renal injury assessment

Following treatment, mice were individually placed in metabolic cages, and spot urine was collected from each mice using the bladder massage method [29], and urine protein level was evaluated by a Coomassie brilliant blue dye-binding assay (Sigma-Aldrich).

Fresh blood was harvested from abdominal aorta, followed by a 15-min centrifugation at 3000 rpm. The blood urea nitrogen (BUN) and creatinine levels in serum were measured using QuantiChrom urea and creatinine assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and a HITACHI-7080 automatic biochemical analyzer.

Renal histopathology

After sacrificing the animals, the kidneys were resected, fixed overnight in 4% paraformaldehyde, paraffin-embedded, and cut into 5-μm sections. Then, the sections were stained with hematoxylin-eosin (HE; Sigma-Aldrich) and visualized using a light microscope

(Olympus, Tokyo, Japan). The tubular and glomerular damages were scored semi-quantitatively on the previously described scoring system by two independent pathologists [30, 31]. Glomerular pathology was evaluated by assessing 60–80 glomeruli per kidney, and each glomerulus was scored on a semiquantitative scale: 0, no change, normal glomerulus; 1, lining of capillaries to Bowman's capsule; 2, accumulation of mesangial matrix in 25% of the glomerulus; 3, accumulation of mesangial matrix in 50% of the glomerulus and capillary obliteration; 4, accumulation of mesangial matrix in 75% of the glomerulus. The tubular injury was scored according to the percentage of damage including atrophy, flattening of proximal tubule epithelial cells, and tubular dilation in 10 randomly selected fields on a scale of 0–5 at a magnification of 200×: 0, no change; 1, < 20% damage; 2, 20–40% damage; 3, 40–60% damage, 4, 60–80% damage; and 5, > 80% damage.

Enzyme-linked immunosorbent assay (ELISA) assay

Kidney tissues were homogenized in ice-cold 0.9% NaCL solution using a glass-Teflon homogenizer and then centrifuged at 3000 *g* for 20 min at 4℃, followed by collection of the supernatants for assessing IFN-γ, IL-6, IL-17 and TNF-α levels using commercially available ELISA kits (Cusabio, Wuhan, China). The anti-dsDNA, IL-1β and IL-18 levels in serum were also estimated *via* ELISA kits. The mouse ELISA kits included IFN-γ (CSB-E04578m), IL-6 (CSB-E04639m), IL-17 (CSB-E04608m), TNF-α (CSB-E04741m) anti-dsDNA antibodies (CSB-E12911m), IL-1β (CSB-E08054m) and IL-18 (CSB-E04609m).

Immunofluorescence staining

Kidney tissues were embedded in OCT compound (MaoKang Biotechnology, Shanghai, China), frozen at –70℃, and then cut into 5 μm-thick sections using a cryostat at –20℃. Subsequently, the sections were blocked in Tris buffered saline with Tween (TBST; pH 7.5; Yeasen, Shanghai, China) containing 1% normal goat serum for 30 min. Thereafter, the sections were incubated with mouse anti-IgG antibodies (1:50; 406601, Biolegend, Beijing, China) or rabbit anti-C3 antibodies (ab97462, 1:100; Abcam, Shanghai, China) overnight at 4℃, followed by incubation with Alexa Fluor 594 goat anti-mouse IgG (ab150116, 1:200;

Abcam) or Alexa Fluor 488 goat anti-rabbit IgG (ab150077, 1:200; Abcam) for 1 h at room temperature. The sections were then placed in phosphate-buffered saline (PBS; Beyotime, Shanghai, China), washed three times for 5 min each, and counterstained with 4',6-diamidino-2-phenylindole (DAPI; Beyotime). The sections were again placed in PBS and washed three times for 5 min each with shaking on a decolorization shaker. When the sections had dried slightly, a small amount of anti-fluorescence quenching agent was dropped onto each slide, and the slide was sealed with resin mounting agent. Finally, the sections were observed under a fluorescence microscope (BX63, Olympus, Tokyo, Japan).

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extract using TRIzol reagent (Sigma-Aldrich), and total RNA (1 μg) was reverse transcribed into cDNA using a PrimeScript RT reagent kit (Takara, Beijing, China). Subsequently, RT-qPCR was performed using the TaqMan Gene Expression Assays Protocol (Thermo Fisher Scientific, Shanghai, China). The amplification conditions included 10 min at 95℃ for initialization, followed by denaturation at 95℃ for 15 s and 60℃ for 30 s with 40 cycles. GAPDH served as the reference gene. Relative mRNA expressions were quantified using the $2^{-\triangle\triangle Ct}$ method [32]. The primer sequences used in this study were listed in Table 1.

Immunohistochemical staining

Samples of kidney cortex and medulla were fixed in 10% neutral-buffered formalin solution (MaoKang Biotechnology) overnight at room temperature, dehydrated, paraffin-embedded, and cut into 3 μm-thick sections. Then, the sections were de-paraffinized to water and incubated in 3% H_2O_2 for 10 min to eliminate endogenous peroxidase activity, followed by antigen retrieval. Subsequently, the sections were soaked in PBS (5 min, two times), blocked with 10% normal goat serum and incubated at room temperature for 30 min. Thereafter, the sections were incubated with primary antibodies against rabbit anti-F4/80 antibodies (ab300421, 1:500; Abcam) and rabbit anti-NLRP3 antibodies (MA5-32255, 1:100; Thermo Fisher Scientific) overnight at 4℃. Next, the sections were washed with PBS and incubated with Alexa Fluor 488 goat anti-rabbit IgG (ab150077, 1:500; Abcam) for 2 h at 37℃. The

HRP-labelled streptavidin (Beyotime, Shanghai, China) was added for incubation at 37℃ for 20 min. The sections were developed with 3',3-diaminobenzidine (Sigma-Aldrich) and counter-stained with hematoxylin (Sigma-Aldrich) for 1 min. The mounted sections were observed under a microscope (CKX31, Olympus) after immersing in 1% ammonia water to return blue in color. Five high-power field views per slice both in cortex and medulla were randomly selected with 400 cells per field of view.

Western blotting

Total protein in kidney tissues was lysed in RIPA lysis buffer (Beyotime) containing protease inhibitor and phosphatase inhibitor (MedChemExpress). After being quantified with a BCA Protein Assay kit (Beyotime), protein samples (30 μg) were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Then, the membranes were blocked with 5% skimmed milk in PBS solution with 0.05% Tween (PBST; Thermo Fisher Scientific) for 1 h at room temperature and incubated with rabbit anti-p-NF-κB p65 antibodies (ab76302, 1:1000; Abcam), rabbit anti-IκB antibodies (ab92700, 1:1000; Abcam), mouse anti-β-actin antibodies (ab8226, 1:1000; Abcam), rabbit anti-NF-κB p65 antibodies (ab32536, 1:2000; Abcam), rabbit anti-histone H3 antibodies (ab1791, 1:1000; Abcam), rabbit anti-NLRP3 antibodies (ab263899, 1:1000; Abcam), rabbit anti-cleaved caspase-1 antibodies (#89332, 1:1000; Cell Signaling Technology, Shanghai, China), and rabbit anti-IL-1β antibodies (ab283818, 1:1000; Abcam) at 4℃ overnight. Subsequently, the membranes were washed with PBST and incubated with corresponding horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Immunoblots were developed using enhanced chemiluminescence reagent (Beyotime). Densitometric analysis was performed using ImageJ software (Bio-Rad, Hercules, CA, USA).

Statistical analysis

The data were analyzed using GraphPad Prism 9.0 (GraphPad, San Diego, CA, USA) and expressed as the mean ± standard deviation (SD). One-way analysis of variance followed by

Tukey *post hoc* test was used for comparisons among groups. $p < 0.05$ was considered statistically significant.

RESULTS

Dioscin alleviates lupus nephritis

To investigate dioscin roles in lupus nephritis, we used NZB/W F1 mice. NZB/W F1 mice had higher proteinuria and serum BUN and creatinine concentrations than WT mice, whereas administration of dioscin or prednisone greatly lowered proteinuria and serum BUN and creatinine levels (Fig. 1B–D). Then, HE staining showed that NZB/W F1 mice presented obvious glomerular and tubular damages, such as glomerular expansion, hypercellularity, interstitial inflammation, crescents, increased mesangial matrix and tubular cast deposition. These severe renal lesions were greatly reduced by administration of dioscin or prednisone (Fig. 1E). The quantitative analyses of glomerular and tubular damage scores demonstrated that compared with the control group, the NZB/W F1 group exhibited significantly higher damage scores. However, dioscin or prednisone treatment remarkably decreased the damage scores in NZB/W F1 mice (Fig. 1F, G). These results demonstrate that dioscin significantly attenuates kidney injury in NZB/W F1 mice.

Dioscin declines serum anti-dsDNA and glomerular IgG and C3 deposits

As shown by ELISA, the lupus-prone NZB/W F1 mice exhibited significantly higher serum anti-dsDNA levels than control animals, whereas administration of dioscin or prednisone decreased them (Fig. 2A). The immunofluorescence staining was performed to assess immune complex deposition of IgG and C3. The fluorescence intensities of antibodies labeling for IgG and C3 greatly increased in NZB/W F1 mice, which were reduced after dioscin or prednisone treatment (Fig. 2B–D).

Dioscin inhibits inflammatory response and macrophage infiltration in lupus-prone mice

The results of RT-qPCR and ELISA demonstrated that mRNA and protein levels of IFN-γ, IL-6, IL-17 and TNF-α expression in homogenates of NZB/W F1 mice kidneys were significantly higher than those in control mice, whereas administration of dioscin decreased

them in NZB/W F1 mice (Fig. 3A, B). Then, we detected macrophage marker expression (F4/80) in the kidney. As shown by immunohistochemistry staining, the NZB/W F1 mice showed profound infiltration of F4/80-positive macrophages in renal tissues, whereas administration of dioscin resulted in a significantly decreased proportion of F4/80-positive inflammatory cells in kidneys of lupus-prone mice (Fig. 3C, D).

Dioscin inactivates NF-κB signaling in kidneys of lupus-prone mice

As western blotting revealed, compared with control mice, NZB/W F1 mice had remarkably elevated protein levels of p-NF-κB p65 and p-IκB, whereas treatment with dioscin effectively downregulated their levels (Fig. 4A–C). Additionally, the nuclear translocation of NF-κB p65 was remarkably inhibited by dioscin in NZB/W F1 mice (Fig. 4D, E).

Dioscin restrains NLRP3 inflammasome activation in kidneys of lupus-prone mice

The concentrations of IL-1β and IL-18 in serum of NZB/W F1 mice were visibly upregulated than those in control mice, whereas treatment with dioscin markedly decreased their levels (Fig. 5A, B). Immunohistochemistry staining results demonstrated that NLRP3 upregulation in NZB/W F1 mice was inhibited after dioscin administration (Fig. 5C, D). Western blotting analysis revealed that the NZB/W F1 mice had notably higher NLRP3, cleaved caspase-1, and IL-1β protein level in kidney homogenates than controls, whereas administration of dioscin reversed these changes (Fig. 5E–H).

Figure 6 presents the schematic diagram based on the results of this study that suggests the mechanisms by which dioscin alleviates lupus nephritis in the mouse model acting through inhibiting NLRP3 inflammasome and NF-κB activation.

DISCUSSION

Lupus nephritis is an immune complex-mediated glomerulonephritis and occurs in approximately 40% of patients diagnosed with SLE within 5 years. Despite great improvements in lupus nephritis outcomes over the past decades, the risk of kidney failure is 22% for all lupus nephritis classes and 44% for class IV lupus nephritis within 15 years of lupus nephritis diagnosis [33]. Therefore, developing novel agents for the treatment of lupus

nephritis is urgent. Dioscin is an active ingredient of Dioscoreaceae herbs, and increasing evidence has shown its anti-inflammatory and renoprotective effects [24, 25, 34]. However, the potential roles and related mechanisms of dioscin in lupus nephritis remain uncertain. We used NZB/W F1 mice to investigate the potential effect of dioscin in lupus nephritis pathologies. Our findings revealed that dioscin attenuated lupus nephritis by decreasing proteinuria, serum BUN and serum creatinine and alleviating glomerular and tubular damage. Mechanistically, dioscin suppressed the activations of NLRP3 inflammasome and NF-κB.

Formation and deposition of immune complexes in blood vessels and renal glomeruli, resulting in complement activation, the synthesis of inflammatory mediators, immune cell infiltration, proteinuria, and renal fibrosis, is critical in lupus nephritis pathophysiology [35]. Macrophages, innate immune cells, are widely distributed and infiltrated in the kidney and regulate renal immune responses [36]. In lupus nephritis patients, the efferocytosis activity of macrophages is reduced, leading to deficient clearance of apoptotic cells, pro-inflammatory polarization of macrophages, secretion of inflammatory factors, and tissue destruction [37]. As reported, TNF-α, IL-6, IL-17 and IFN-γ are involved in glomerulonephritis pathogenesis [38]. Herein, elevated anti-dsDNA, TNF-α, IL-6, IL-17, and IFN-γ levels, increased glomerular IgG and C3 deposits, and renal infiltration of macrophages were observed in lupus-prone mice. Dioscin can alleviate IgA nephropathy by inhibiting excessive activation of IgA-secreting cells [39]. Additionally, dioscin has been found to inhibit macrophage M1 polarization and macrophage-derived inflammation, thereby ameliorating murine ulcerative colitis [40], inflammatory bowel disease [41] and pulmonary fibrosis [42]. The current study revealed that dioscin downregulated serum anti-dsDNA concentrations and decreased glomerular IgG and C3 deposition. Additionally, dioscin also decreased TNF-α, IL-6, IL-17 and IFN-γ levels and inhibited macrophage infiltration in NZB/W F1 mice.

The NLRP3 inflammasome is activated in macrophages isolated from lupus patients [43]. Inflammatory stimuli can activate NLRP3 inflammasome and subsequently induce caspase-1 activation and IL-1β and IL-18 release [44]. Blockade of the NLRP3 inflammasome is found to attenuate lupus nephritis in MRP/lpr mice [16]. NF-κB is a transcription factor. Once the IκB kinase complexes phosphorylate IκB, nuclear translocation of NF-κB appears, initiating

inflammation [45]. In the current study, NZB/W F1 mice exhibited activation of NLRP3 inflammasome. Additionally, increased NF-κB p65 and IκB phosphorylation and elevated nuclear NF-κB p65 expression were observed in NZB/W F1 mice. These findings revealed NLRP3 inflammasome and NF-κB activations in NZB/W F1 mice. Dioscin was shown to prevent experimental gouty arthritis, mastitis, colitis, periodontitis and diabetes cognitive dysfunction by inhibiting NLRP3 inflammasome activation [27, 46–49]. Additionally, dioscin can ameliorate renal fibrosis, diabetic nephropathy and inflammatory kidney injury by inactivating NF-κB signaling [24, 25, 50]. Our study demonstrated that dioscin inactivated NLRP3 inflammasome and NF-κB signaling in lupus-prone NZB/W F1 mice.

In conclusion, dioscin ameliorates lupus nephritis through inactivating NLRP3 inflammasome and NF-κB. However, there are some limitations to this study. First, the MRL/lpr mouse model recapitulates many of the clinical manifestations and immune dysregulation observed in human SLE [51]. Experiments should also be conducted in MRL/lpr mice. Second, dioscin roles in podocyte injury, autophagy and oxidative stress in lupus nephritis should be detected. Third, the safety of dioscin in clinical practice remains unknown. Despite these limitations, our findings support for the clinical translation of dioscin as novel treatments for lupus nephritis.

ARTICLE INFORMATION AND DECLARATIONS

Data availability statement

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics statement

Laboratory procedures were approved by Ethics Committee of Wuhan Sixth Hospital. All the animal care was performed according to the Guide for Care and Use of Laboratory Animals.

Author contributions

Yaling Xu conceived and designed the experiments. Yaling Xu and Han Li carried out the experiments. Yaling Xu and Han Li analyzed the data. Yaling Xu and Han Li drafted the manuscript. All authors agreed to be accountable for all aspects of the work. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing interests.

REFERENCES

1. Tian J, Zhang D, Yao Xu, et al. Global epidemiology of systemic lupus erythematosus: a comprehensive systematic analysis and modelling study. Ann Rheum Dis. 2023; 82(3): 351–356, doi: 10.1136/ard-2022-223035, indexed in Pubmed: 36241363.
2. Hanly JG, O'Keeffe AG, Su Li, et al. The frequency and outcome of lupus nephritis: results from an international inception cohort study. Rheumatology (Oxford). 2016; 55(2): 252–262, doi: 10.1093/rheumatology/kev311, indexed in Pubmed: 26342222.
3. Li D, Shi G, Wang J, et al. Baicalein ameliorates pristane-induced lupus nephritis via activating Nrf2/HO-1 in myeloid-derived suppressor cells. Arthritis Res Ther. 2019; 21(1): 105, doi: 10.1186/s13075-019-1876-0, indexed in Pubmed: 31023362.
4. Kudose S, Santoriello D, Bomback A, et al. Sensitivity and specificity of pathologic findings to diagnose lupus nephritis. Clin J Am Soc Nephrol. 2019; 14(11): 1605–1615, doi: 10.2215/cjn.01570219, indexed in Pubmed: 31653670.

5. Chalmers SA, Chitu V, Ramanujam M, et al. Therapeutic targeting of macrophages in lupus nephritis. Discov Med. 2015; 20(108): 43–49, indexed in Pubmed: 26321086.

6. Yung S, Cheung KF, Zhang Q, et al. Anti-dsDNA antibodies bind to mesangial annexin II in lupus nephritis. J Am Soc Nephrol. 2010; 21(11): 1912–1927, doi: 10.1681/ASN.2009080805, indexed in Pubmed: 20847146.

7. Anders HJ, Saxena R, Zhao MH, et al. Lupus nephritis. Nat Rev Dis Primers. 2020; 6(1): 7, doi: 10.1038/s41572-019-0141-9, indexed in Pubmed: 31974366.

8. Fu J, Wu H. Structural mechanisms of NLRP3 inflammasome assembly and activation. Annu Rev Immunol. 2023; 41: 301–316, doi: 10.1146/annurev-immunol-081022-021207, indexed in Pubmed: 36750315.

9. Latz E, Xiao TS, Stutz A. Activation and regulation of the inflammasomes. Nat Rev Immunol. 2013; 13(6): 397–411, doi: 10.1038/nri3452, indexed in Pubmed: 23702978.

10. Huang T, Yin H, Ning W, et al. Expression of inflammasomes NLRP1, NLRP3 and AIM2 in different pathologic classification of lupus nephritis. Clin Exp Rheumatol. 2020; 38(4): 680–690, indexed in Pubmed: 31694740.

11. Honarpisheh M, Desai J, Marschner JA, et al. Regulated necrosis-related molecule mRNA expression in humans and mice and in murine acute tissue injury and systemic autoimmunity leading to progressive organ damage, and progressive fibrosis. Biosci Rep. 2016; 36(6), doi: 10.1042/BSR20160336, indexed in Pubmed: 27811014.

12. Bonomini F, Dos Santos M, Veronese FV, et al. NLRP3 Inflammasome Modulation by Melatonin Supplementation in Chronic Pristane-Induced Lupus Nephritis. Int J Mol Sci. 2019; 20(14), doi: 10.3390/ijms20143466, indexed in Pubmed: 31311094.

13. Fu R, Guo C, Wang S, et al. Podocyte activation of NLRP3 inflammasomes contributes to the development of proteinuria in lupus nephritis. Arthritis Rheumatol. 2017; 69(8): 1636–1646, doi: 10.1002/art.40155, indexed in Pubmed: 28544564.

14. Fu R, Xia Y, Li M, et al. Pim-1 as a therapeutic target in lupus nephritis. Arthritis Rheumatol. 2019; 71(8): 1308–1318, doi: 10.1002/art.40863, indexed in Pubmed: 30791224.

15. Wu CY, Hua KF, Chu CL, et al. Tris DBA ameliorates accelerated and severe lupus nephritis in mice by activating regulatory t cells and autophagy and inhibiting the NLRP3 inflammasome. J Immunol. 2020; 204(6): 1448–1461, doi: 10.4049/jimmunol.1801610, indexed in Pubmed: 32060137.

16. Zhao J, Zhang H, Huang Y, et al. Bay11-7082 attenuates murine lupus nephritis via inhibiting NLRP3 inflammasome and NF-κB activation. Int Immunopharmacol. 2013; 17(1): 116–122, doi: 10.1016/j.intimp.2013.05.027, indexed in Pubmed: 23770281.

17. Li Q, Verma IM. NF-kappaB regulation in the immune system. Nat Rev Immunol. 2002; 2(10): 725–734, doi: 10.1038/nri910, indexed in Pubmed: 12360211.

18. Williams LM, Gilmore TD. Looking down on NF-κB. Mol Cell Biol. 2020; 40(15), doi: 10.1128/MCB.00104-20, indexed in Pubmed: 32393609.

19. Zheng L, Sinniah R, Hsu SIH. Pathogenic role of NF-kappaB activation in tubulointerstitial inflammatory lesions in human lupus nephritis. J Histochem Cytochem. 2008; 56(5): 517–529, doi: 10.1369/jhc.7A7368.2008, indexed in Pubmed: 18285351.

20. Li X, Wang M, Hong H, et al. Sophocarpine attenuates murine lupus nephritis via inhibiting NLRP3 inflammasome and NF-κB activation. Immunol Res. 2018; 66(4): 521–527, doi: 10.1007/s12026-018-9012-9, indexed in Pubmed: 30047025.

21. Su B, Ye H, You X, et al. Icariin alleviates murine lupus nephritis via inhibiting NF-κB activation pathway and NLRP3 inflammasome. Life Sci. 2018; 208: 26–32, doi: 10.1016/j.lfs.2018.07.009, indexed in Pubmed: 30146016.

22. Tao X, Yin L, Xu L, et al. Dioscin: A diverse acting natural compound with therapeutic potential in metabolic diseases, cancer, inflammation and infections. Pharmacol Res. 2018; 137: 259–269, doi: 10.1016/j.phrs.2018.09.022, indexed in Pubmed: 30315966.

23. Qi M, Zheng L, Qi Y, et al. Dioscin attenuates renal ischemia/reperfusion injury by inhibiting the TLR4/MyD88 signaling pathway via up-regulation of HSP70. Pharmacol Res. 2015; 100: 341–352, doi: 10.1016/j.phrs.2015.08.025, indexed in Pubmed: 26348276.

24. Wang Y, Liu P, Ma G, et al. Mechanism of dioscin ameliorating renal fibrosis through NF-κB signaling pathway-mediated inflammatory response. Mol Med Rep. 2023; 27(4), doi: 10.3892/mmr.2023.12980, indexed in Pubmed: 36960871.

25. Cai S, Chen J, Li Y. Dioscin protects against diabetic nephropathy by inhibiting renal inflammation through TLR4/NF-κB pathway in mice. Immunobiology. 2020; 225(3): 151941, doi: 10.1016/j.imbio.2020.151941, indexed in Pubmed: 32359778.

26. Zhang P, Lei X, Ou L, et al. Dioscin ameliorates silica-aggravated systemic lupus erythematosus via suppressing apoptosis and improving LC3-associated phagocytosis in MRL/lpr mice. Int Immunopharmacol. 2023; 116: 109814, doi: 10.1016/j.intimp.2023.109814, indexed in Pubmed: 36773568.

27. Jiang X, Ding X, Wei J, et al. Dioscin alleviates periodontitis by inhibiting NLRP3 inflammasome activation via regulation of k homeostasis and mitochondrial function. Int J Biol Sci. 2024; 20(4): 1375–1388, doi: 10.7150/ijbs.85851, indexed in Pubmed: 38385066.

28. Liang CL, Lu W, Qiu F, et al. Paeoniflorin ameliorates murine lupus nephritis by increasing CD4Foxp3 Treg cells via enhancing mTNFα-TNFR2 pathway. Biochem Pharmacol. 2021; 185: 114434, doi: 10.1016/j.bcp.2021.114434, indexed in Pubmed: 33513343.

29. Qi Q, Li H, Lin ZM, et al. (5R)-5-hydroxytriptolide ameliorates anti-glomerular basement membrane glomerulonephritis in NZW mice by regulating Fcγ receptor signaling. Acta Pharmacol Sin. 2018; 39(1): 107–116, doi: 10.1038/aps.2017.88, indexed in Pubmed: 28880016.

30. Jia Y, He J, Wang L, et al. Dapagliflozin aggravates renal injury via promoting gluconeogenesis in db/db mice. Cell Physiol Biochem. 2018; 45(5): 1747–1758, doi: 10.1159/000487783, indexed in Pubmed: 29495021.

31. Kökény G, Fang L, Révész C, et al. The Effect of Combined Treatment with the (Pro)Renin Receptor Blocker HRP and Quinapril in Type 1 Diabetic Rats. Kidney Blood Press Res. 2017; 42(1): 109–122, doi: 10.1159/000471915, indexed in Pubmed: 28359068.

32. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25(4): 402–408, doi: 10.1006/meth.2001.1262, indexed in Pubmed: 11846609.

33. Tektonidou MG, Dasgupta A, Ward MM. Risk of end-stage renal disease in patients with lupus nephritis, 1971–2015: a systematic review and Bayesian meta-analysis. Arthritis Rheumatol. 2016; 68(6): 1432–1441, doi: 10.1002/art.39594, indexed in Pubmed: 26815601.

34. Qiao Y, Xu L, Tao X, et al. Protective effects of dioscin against fructose-induced renal damage via adjusting Sirt3-mediated oxidative stress, fibrosis, lipid metabolism and inflammation. Toxicol Lett. 2018; 284: 37–45, doi: 10.1016/j.toxlet.2017.11.031, indexed in Pubmed: 29197622.

35. Tsokos GC. Systemic lupus erythematosus. N Engl J Med. 2011; 365(22): 2110–2121, doi: 10.1056/NEJMra1100359, indexed in Pubmed: 22129255.

36. Richoz N, Tuong ZK, Loudon KW, et al. Distinct pathogenic roles for resident and monocyte-derived macrophages in lupus nephritis. JCI Insight. 2022; 7(21), doi: 10.1172/jci.insight.159751, indexed in Pubmed: 36345939.

37. Ma C, Xia Yu, Yang Q, et al. The contribution of macrophages to systemic lupus erythematosus. Clin Immunol. 2019; 207: 1–9, doi: 10.1016/j.clim.2019.06.009, indexed in Pubmed: 31255802.

38. Kurts C, Panzer U, Anders HJ, et al. The immune system and kidney disease: basic concepts and clinical implications. Nat Rev Immunol. 2013; 13(10): 738–753, doi: 10.1038/nri3523, indexed in Pubmed: 24037418.

39. Lin L, Shen J, Wu X, et al. Dioscin mediated IgA nephropathy alleviation by inhibiting B cell activation in vivo and decreasing galactose-deficient IgA1 production in vitro. J Vis Exp. 2023(200), doi: 10.3791/65719, indexed in Pubmed: 37902367.

40. Wu MM, Wang QM, Huang BY, et al. Dioscin ameliorates murine ulcerative colitis by regulating macrophage polarization. Pharmacol Res. 2021; 172: 105796, doi: 10.1016/j.phrs.2021.105796, indexed in Pubmed: 34343656.

41. Shi L, Zhang P, Jin R, et al. Dioscin ameliorates inflammatory bowel disease by up-regulating miR-125a-5p to regulate macrophage polarization. J Clin Lab Anal. 2022; 36(6): e24455, doi: 10.1002/jcla.24455, indexed in Pubmed: 35524480.

42. Li C, Lu Y, Du S, et al. Dioscin exerts protective effects against crystalline silica-induced pulmonary fibrosis in mice. Theranostics. 2017; 7(17): 4255–4275, doi: 10.7150/thno.20270, indexed in Pubmed: 29158824.

43. Kahlenberg JM, Carmona-Rivera C, Smith CK, et al. Neutrophil extracellular trap-associated protein activation of the NLRP3 inflammasome is enhanced in lupus macrophages. J Immunol. 2013; 190(3): 1217–1226, doi: 10.4049/jimmunol.1202388, indexed in Pubmed: 23267025.

44. Liu X, Bao C, Hu D. Elevated interleukin-18 and skewed Th1:Th2 immune response in lupus nephritis. Rheumatol Int. 2012; 32(1): 223–229, doi: 10.1007/s00296-010-1609-9, indexed in Pubmed: 20963419.

45. Ding Yi, Chen Q. The NF-κB Pathway: a Focus on Inflammatory Responses in Spinal Cord Injury. Mol Neurobiol. 2023; 60(9): 5292–5308, doi: 10.1007/s12035-023-03411-x, indexed in Pubmed: 37286724.

46. Han J, Shi G, Li W, et al. Preventive effect of dioscin against monosodium urate-mediated gouty arthritis through inhibiting inflammasome NLRP3 and TLR4/NF-κB

signaling pathway activation: an in vivo and in vitro study. J Nat Med. 2021; 75(1): 37–47, doi: 10.1007/s11418-020-01440-7, indexed in Pubmed: 32761488.

47. Ran X, Yan Z, Yang Y, et al. Dioscin improves pyroptosis in LPS-induced mice mastitis by activating AMPK/Nrf2 and inhibiting the NF-κB signaling pathway. Oxid Med Cell Longev. 2020; 2020: 8845521, doi: 10.1155/2020/8845521, indexed in Pubmed: 33488936.

48. Cai J, Liu J, Fan P, et al. Dioscin prevents DSS-induced colitis in mice with enhancing intestinal barrier function and reducing colon inflammation. Int Immunopharmacol. 2021; 99: 108015, doi: 10.1016/j.intimp.2021.108015, indexed in Pubmed: 34339962.

49. Lu Z, Yao Y, Wang J, et al. Dioscin ameliorates diabetes cognitive dysfunction via adjusting P2X7R/NLRP3 signal. Int Immunopharmacol. 2021; 101(Pt B): 108314, doi: 10.1016/j.intimp.2021.108314, indexed in Pubmed: 34785142.

50. Qi M, Yin L, Xu L, et al. Dioscin alleviates lipopolysaccharide-induced inflammatory kidney injury via the microRNA let-7i/TLR4/MyD88 signaling pathway. Pharmacol Res. 2016; 111: 509–522, doi: 10.1016/j.phrs.2016.07.016, indexed in Pubmed: 27431331.

51. Perry D, Sang A, Yin Y, et al. Murine models of systemic lupus erythematosus. J Biomed Biotechnol. 2011; 2011: 271694, doi: 10.1155/2011/271694, indexed in Pubmed: 21403825.

Table 1. Sequences of primers used for reverse transcription-quantitative PCR

Gene (mice)	**Sequence (5'→3')**
IFN-γ forward	AGCAAGGCGAAAAAGGATGC
IFN-γ reverse	TCATTGAATGCTTGGCGCTG
IL-6 forward	TGTATGAACAACGATGATGCAC
IL-6 reverse	TGGTACTCCAGAAGACCAGAGG
IL-17 forward	ACCGCAATGAAGACCCTGAT
IL-17 reverse	CAGGATCTCTTGCTGGATGAGA
TNF-α forward	CGAGTGACAAGCCTGTAGCC
TNF-α reverse	GAGAACCTGGGAGTAGACAAGG
GAPDH forward	TCCGCCCCTTCTGCCGATG
GAPDH reverse	CACGGAAGGCCATGCCAGTGA

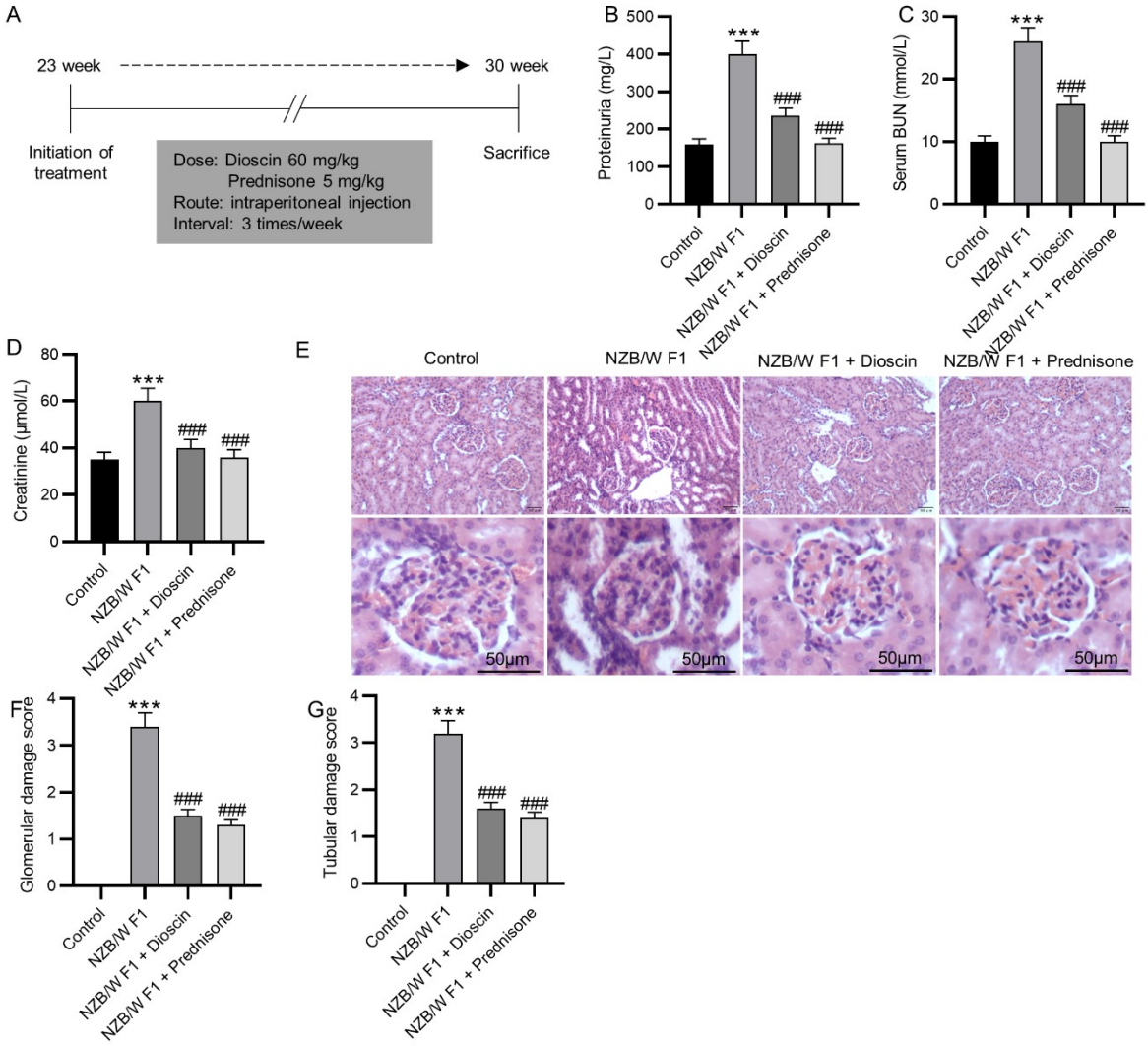

Figure 1. Dioscin alleviates the pathological features of lupus nephritis in NZB/W F1 mice. **A.** Treatment schedule for NZB/W F1 mice with PBS (vehicle), dioscin (60 mg/kg) and prednisone (5 mg/kg). **B–D.** The levels of proteinuria, serum BUN and creatinine in the control, the NZB/W F1, the dioscin and the prednisone groups. **E.** Representative images of HE staining in the renal tissues. **F, G.** Quantitative analysis of glomerular and tubular damage scores in renal tissues. There were five mice used in each group. The data indicated the mean SD of three independent experiments. $^{***}P < 0.001$ *vs.* control group; $^{###}P < 0.001$ *vs.* NZB/W F1 group.

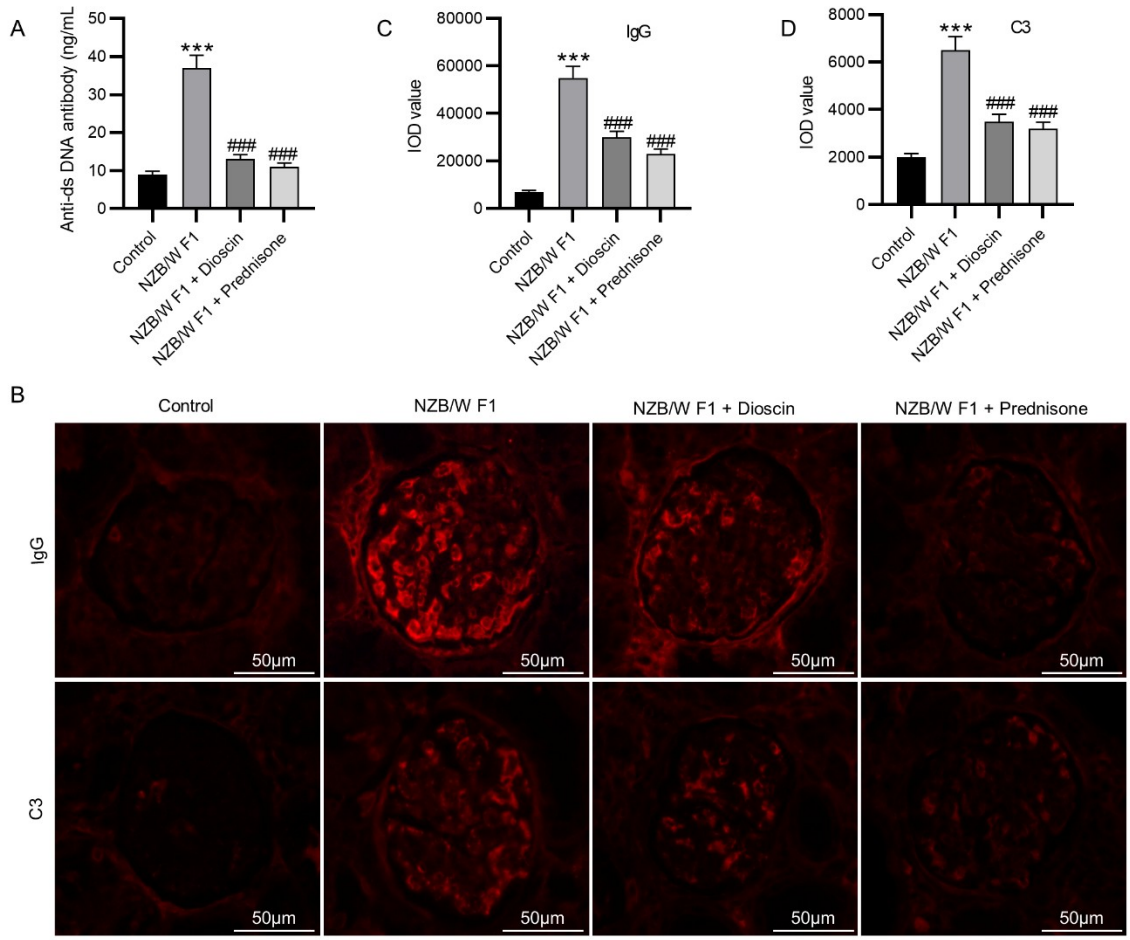

Figure 2. Dioscin reduces serum anti-dsDNA level and glomerular deposition of IgG and C3 in NZB/W F1 mice. **A.** The levels of serum anti-dsDNA antibodies were measured by ELISA. **B.** Representative immunofluorescence staining images for IgG and C3 deposition in the kidney glomeruli. **C, D.** Quantitative analysis of IgG and C3 deposition in the glomeruli, presented as the IOD value (integrated optical density value). The data indicated the mean SD of three independent experiments, n = 5 in each group. $^{***}P < 0.001$ *vs*. control group; $^{###}P < 0.001$ *vs*. NZB/W F1 group.

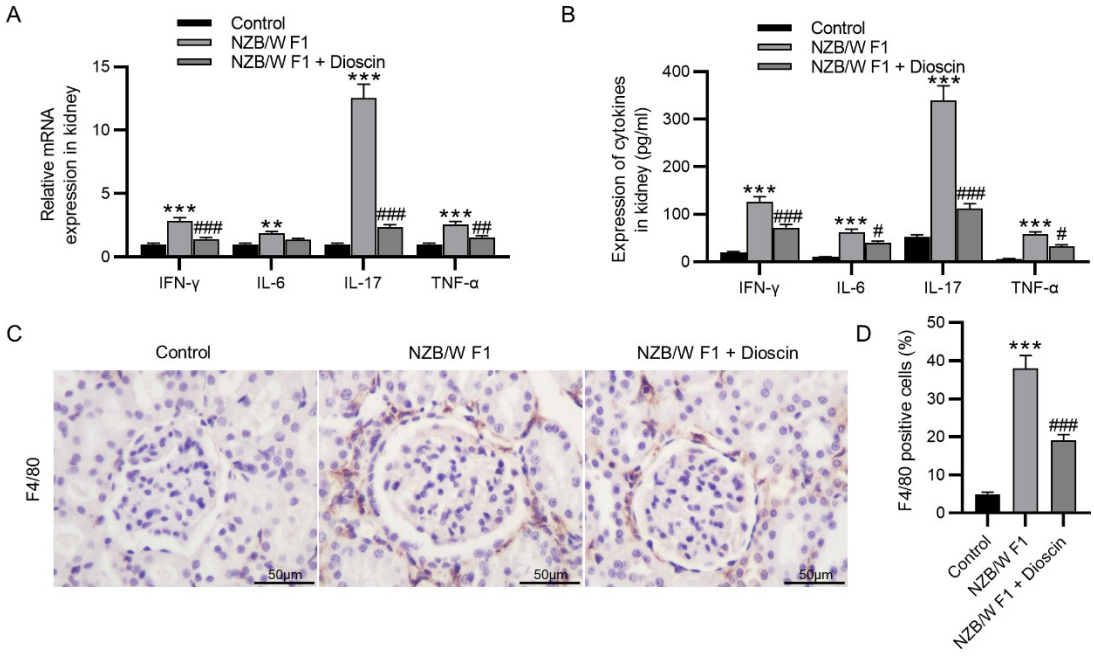

Figure 3. Dioscin inhibits the inflammatory response and infiltration of macrophages into the kidneys of NZB/W F1 mice. **A, B.** The mRNA and protein levels of IFN-γ, IL-6, IL-17 and TNF-α in mouse kidney tissues were estimated by RT-qPCR and ELISA. **C, D.** Macrophage infiltration was detected by immunohistochemistry staining. n = 5 in each group. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* control group; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ *vs.* NZB/W F1 group.

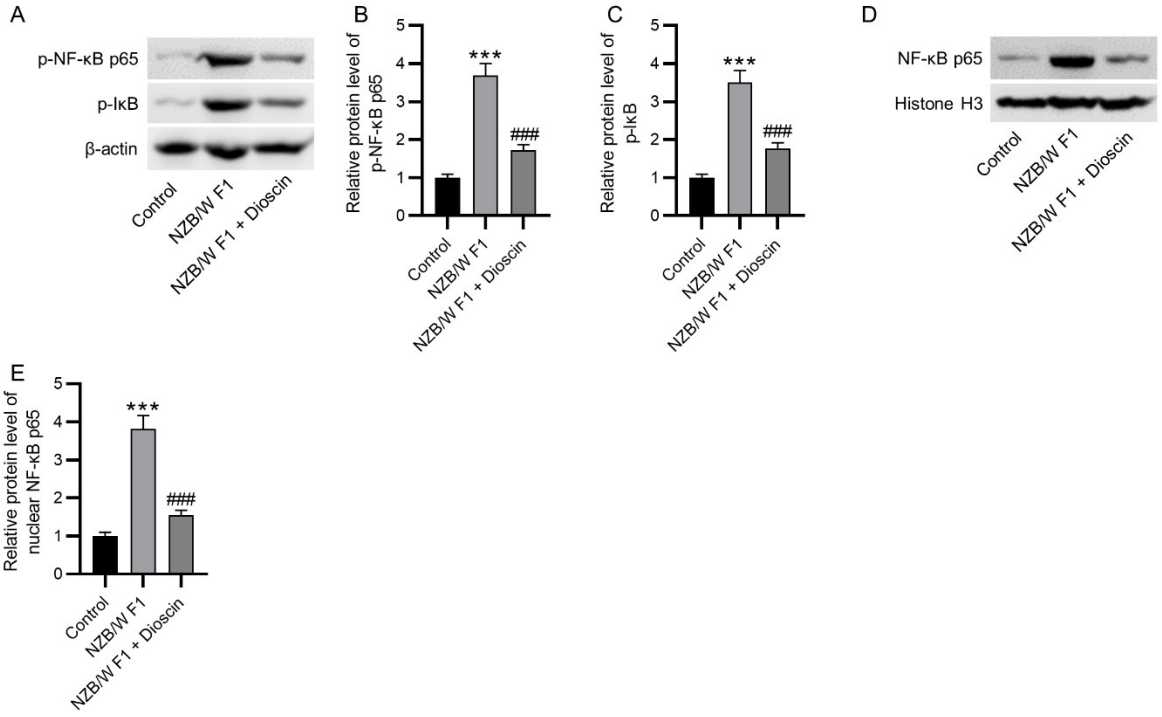

Figure 4. Dioscin inactivates NF-κB signaling in the kidneys of NZB/W F1 mice.

A–C. The protein levels of p-NF-κB p65 and p-IκB in renal tissues were measured by western blotting. **D, E.** Nuclear NF-κB p65 was determined by western blotting. n=5 in each group. $^{***}P < 0.001$ *vs.* control group; $^{###}P < 0.001$ *vs.* NZB/W F1 group.

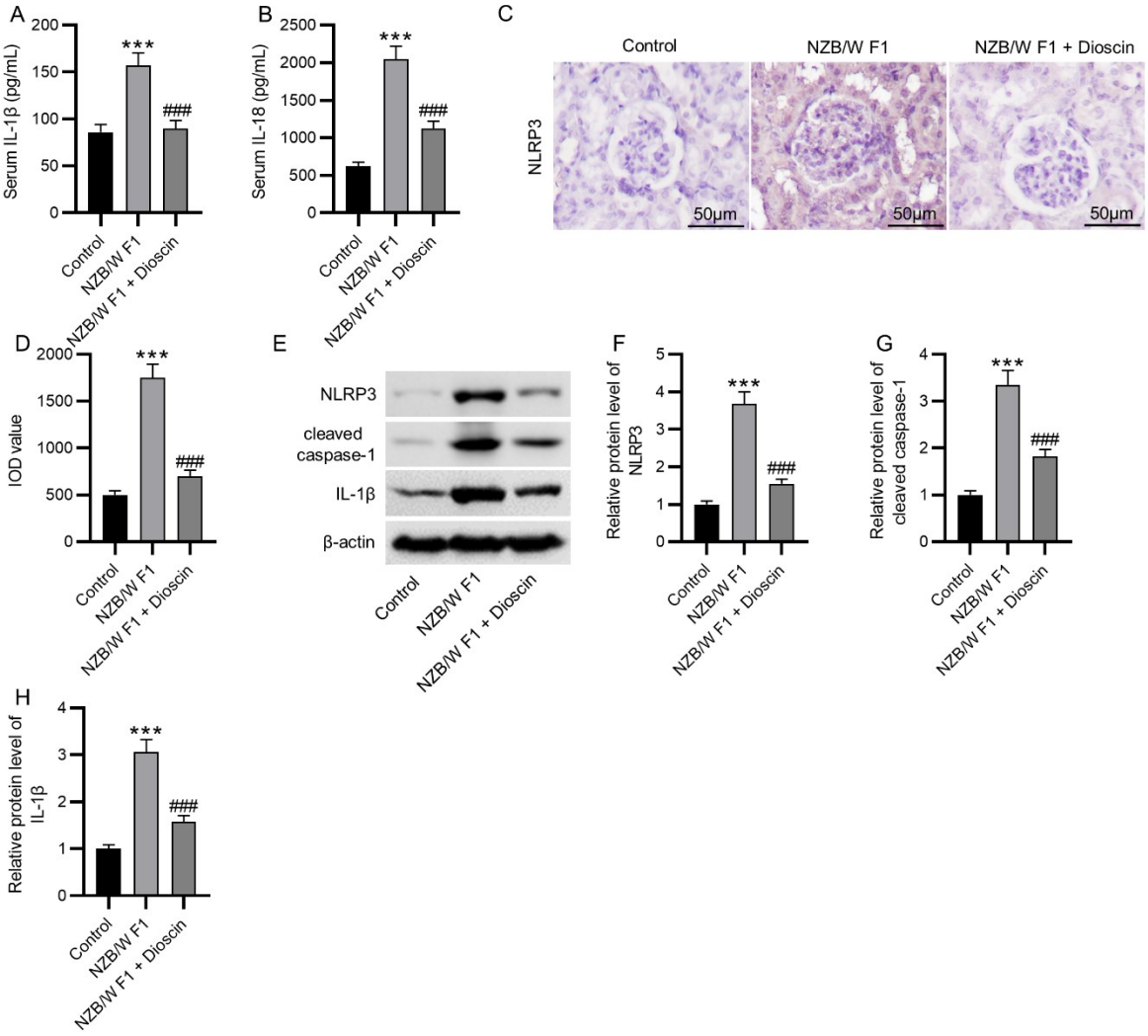

Figure 5. Dioscin restrains NLRP3 inflammasome activation in the kidneys of NZB/W F1 mice. **A, B.** The serum levels of IL-1β and IL-18 were estimated by ELISA. **C, D.** Immunohistochemistry staining of NLRPS in kidney tissues. **E–H.** Western blotting to measure the protein levels of NLRP3, cleaved caspase-1 and IL-1β. n = 5 in each group. $^{***}P < 0.001$ *vs.* control group; $^{###}P < 0.001$ *vs.* NZB/W F1 group.

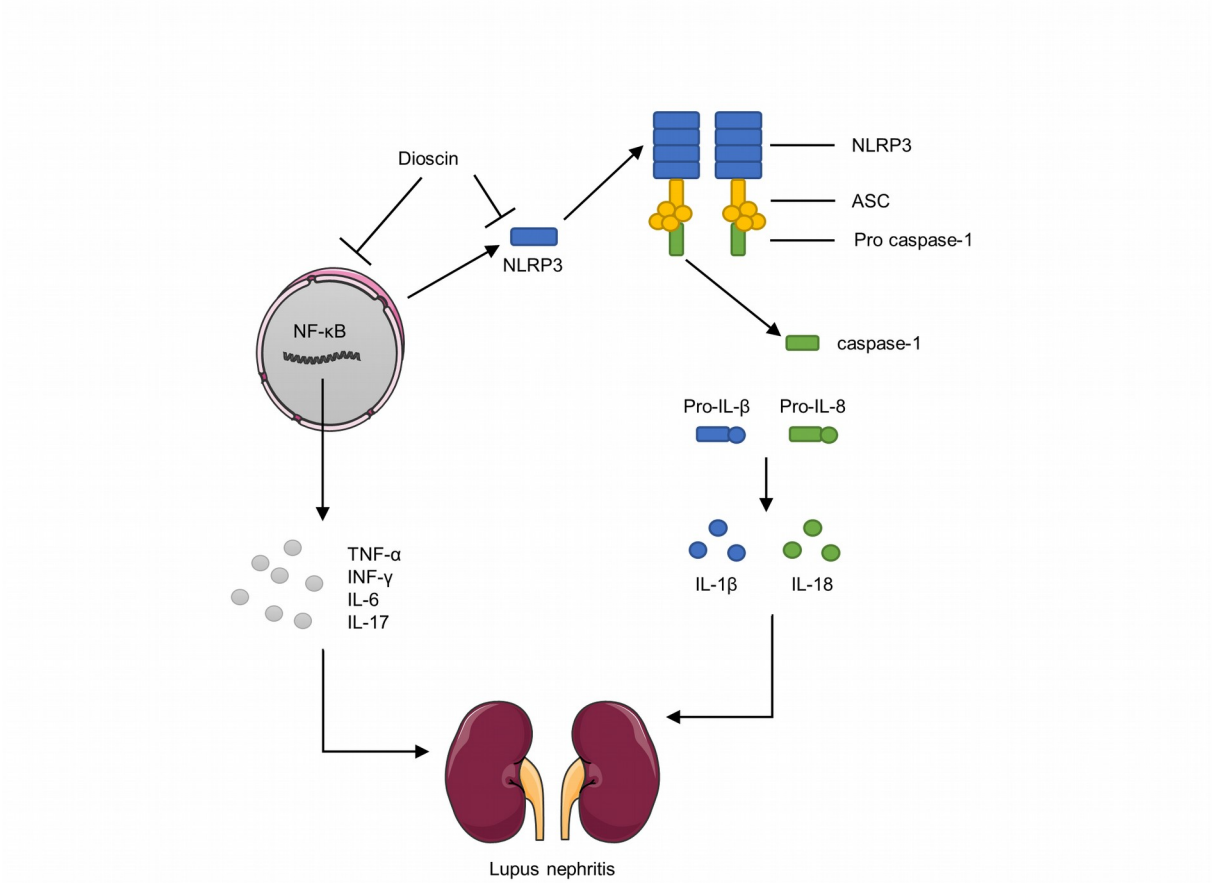

Figure 6. Schematic diagram depicts the mechanisms by which dioscin alleviates lupus nephritis. Dioscin ameliorates lupus nephritis through inhibiting NLRP3 inflammasome and NF-κB activation.