



Preventive effect of dioscin against monosodium urate-mediated gouty arthritis through inhibiting inflammasome NLRP3 and TLR4/NF- κ B signaling pathway activation: an in vivo and in vitro study

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Abstract

Monosodium urate (MSU)-mediated inflammation is closely related to gouty arthritis (GA). Dioscin, an active ingredient, has been reported to possess anti-inflammatory property. Nevertheless, the role of dioscin in GA and the underlying mechanism have not been fully understood. In the present study, we investigated the anti-inflammatory effect of dioscin on MSU-induced GA through in vivo and in vitro experiments. Histopathological analysis showed that dioscin alleviated the severity of GA concomitant with the lowered uric acid and creatinine levels. Moreover, the increasing IL-1 β , IL-6, and TNF- α levels induced by MSU were decreased via administration of dioscin in mice and human synoviocytes. Western blotting results suggested that dioscin inhibited the activation of NLRP3 through down-regulating the protein expressions of NLRP3, apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), cleaved-caspase-1, as well as IL-1 β . In addition, TLR4, myeloid differentiation primary response gene 88 (MyD88), p-IKK β , p-p65, and NF- κ B p65 in nuclei levels were significantly reduced by dioscin. Importantly, dioscin remarkably lowered the NF- κ B p65-DNA activity in MSU-treated mice utilizing electrophoretic mobility shift assay (EMSA) analysis. Taken together, dioscin had a protective effect against MSU-initiated inflammatory response via repressing the production of inflammatory cytokines and the activation of inflammasome NLRP3 and TLR4/NF- κ B signaling pathway. The above findings revealed that dioscin could be a potential drug for the treatment of GA.

Keywords Dioscin · Gouty arthritis · NLRP3 · TLR4/NF- κ B signaling pathway

Introduction

Gouty arthritis (GA), the most common inflammatory arthritis in adults, is characterized by elevated uric acid content in the body and the deposition of uric acid crystals mainly in and around the joint space accompanied by acute inflammation [1, 2]. Epidemiological studies reveal that gout

has become an ordinary and frequently-occurring disease throughout the world, and it is a metabolic disease second only to diabetes in China [3]. In gout, monosodium urate crystals (MSU) can be phagocytosed by macrophages, thus mediating the release of pro-inflammatory cytokines such as interleukin (IL)-1 β and IL-6 and promoting the activation of inflammasome [4]. In turn, the release of these factors is capable of facilitating synovial cells to participate in inflammatory response of gout. Though anti-inflammatory therapies including NSAIDs, colchicines, as well as corticosteroids have been employed for the treatment of GA, as a result of adverse effects (significant toxicities and hyperkalaemia), their use is limited [1]. Hence, finding safe and effective drugs for preventing and curing GA is particularly necessary.

Dioscorea containing *Dioscorea septemioba* and *Dioscorea spongiosa* possesses the functions of removing dampness, clearing turbidity, and removing wind and pain. It has been intensively utilized for treating homosexual

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urinary leaching manifested by chyluria, joint stiffness, as well as rheumatic arthralgia [5]. The earlier studies reported that *Dioscorea spongiosa* had curative roles in osteoporosis through in vivo and in vitro investigations [6, 7]. The previous work indicated that dioscin extracted from *Dioscorea spongiosa* could reduce blood uric acid and effectively treat hyperuricemia [8]. Furthermore, *Dioscorea* is able to inhibit lipopolysaccharide-induced inflammation in RAW264.7 murine macrophages, and lower the level of uric acid and kidney injury in rat and mouse models of hyperuricemia [9–11]. Lu et al. [12] pointed out that Rhizoma *Dioscorea nipponicae* might have therapeutic effects on GA via suppressing monosodium urate (MSU)-induced inflammatory response in rats and IL-1 β -mediated synovial cell proliferation. It was documented that dioscin relieved arthritis triggered by collagen through inhibition of Th17 cell response [13]. What is more, dioscin exhibited a good protective effect against cartilage destruction in an osteoarthritis rat model induced by monosodium iodoacetate [14]. Therefore, it is worthy of thoroughly studying the anti-inflammatory role of dioscin in GA via in vivo and in vitro assays.

In the current study, we investigated the effect of dioscin on MSU-induced inflammation in mice (an animal model for GA) and human synoviocytes (HS). The level of inflammatory NLR family pyrin domain containing three (NLRP3) was assessed by immunohistochemistry staining and western blotting. Moreover, Toll-like receptor 4 (TLR4) and nuclear factor- κ B (NF- κ B) pathways tightly involved in inflammatory response were evaluated using western blotting. Our experiment results demonstrated that dioscin reduced inflammation possibly through inhibition of the activation of NLRP3 and TLR4/NF- κ B signaling pathway, which was helpful to cure GA.

Materials and methods

Materials

Dioscin was purchased from Shanghai yuanye Bio-Technology Co., Ltd (B21176, China). MSU was supplied by Sigma (U2875, USA). Eight-week-old male C57BL/6 mice (20–22 g) were obtained from Liaoning Changsheng biotechnology Co., Ltd (Shenyang, China). Primary human synoviocytes (HS) were provided by Procell Life Science and Technology Co., Ltd (Wuhan, China).

Mouse models

Eight-week-old male C57BL/6 mice were randomly divided into four groups: control group, MSU group, MSU + L-Dioscin group, and MSU + H-Dioscin group. Mice were administrated by dioscin (50 or 100 mg/kg) by

gavage every day, and/or MSU (25 mg/mL diluted in PBS) was injected into the right ankle of the mouse on the 8th day after intragastric administration for 1 h. Then, swollen right palm in mice was photographed at 0, 24, and 48 h. Not only were the mice euthanized with 200 mg/kg of pentobarbital, but also both blood and joint synovial tissue samples were collected for subsequent experiments. The handling of mice and experimental procedures in our study were carried out in the light of the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH, no. 85-23, 1996), and were approved by the Animal Ethics Committee of the First Affiliated Hospital, Heilongjiang University of Chinese Medicine.

Measurement of uric acid and creatinine in serum

We utilized the corresponding assay kits (Nanjing Jiancheng Bioengineering Institute, China) to evaluate uric acid and creatinine contents in serum of mice with and without treatment in accordance with the manufacturers' instructions.

Histopathological assessment

The fixed synovial tissues were dehydrated and embedded a mixture containing xylene as well as paraffin (60 °C, 2 h), and then cut into 5- μ m-thickness slices. The slices were stained with hematoxylin–eosin (H&E) and observed under a microscope (BX53, OLUMPUS, Japan).

Cell culture and treatment

HS were cultured with dedicated medium (CM-H094, Procell) in an incubator (37 °C, 5% CO₂). Cells were administrated by dioscin (15 ng/mL) or CLI-095 (5 μ mol/L, TAK-242, TLR4 inhibitor, Shanghai yuanye) for 24 h, and then treated with MSU (100 μ g/mL) for 12 h.

Cell viability detection

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, KGA311) was provided by KeyGEN BioTECH (Nanjing, China). Dimethyl sulfoxide (DMSO, ST038) was purchased from Beyotime Biotechnology (Shanghai, China). After treatment with dioscin or CLI-095 and/or MSU, MTT (0.5 mg/mL) was added to treated cells and reacted for 5 h under a CO₂ incubator at 37 °C. The supernatant was removed from each well, and 150 μ L of DMSO was added. After reaction for 10 min in the dark, the absorbance was detected at 570 nm using a microplate reader (ELX-800, Biotek, USA).

Enzyme-linked immunosorbent assay (ELISA)

The levels of IL-1 β , IL-6, and TNF- α were assessed in mouse synovium tissues and cell supernatant by ELISA kits following the manufacturers' recommendation, respectively. IL-1 β kit (SEA563Mu; SEA563Hu), IL-6 kit (SEA079Mu; SEA079Hu), and TNF- α kit (SEA133Hu) were purchased from Uscn (Wuhan, China). The mouse TNF- α kit (EK0527) was supplied by Boster Biological Technology Co., Ltd (Wuhan, China).

Immunohistochemistry staining for NLRP3 in synovium tissues

The sections were deparaffinized and incubated with 3% H₂O₂ at room temperature (RT) for 15 min. After being washed in PBS three times, the slices were blocked with goat serum (SL038, Solarbio, China) at RT for 15 min. After being washed with PBS, the sections were cultured with rabbit NLRP3 antibody (dilution 1:100 in PBS, A12694, abclonal, China) at 4 °C overnight. Subsequently, the slices were incubated with HRP-labeled goat anti-rabbit IgG (dilution 1: 500 in PBS, #31460, Thermo Fisher Scientific, USA) (37 °C, 1 h), treated with 3,3-diaminobenzidine (DAB, DA1010, Solarbio), and counterstained with hematoxylin (H8070, Solarbio) for 3 min. Images were photographed by a microscope.

Immunofluorescence assay for NF- κ B p65 and NLRP3 in cells

Treated cells were fixed in 4% paraformaldehyde for 15 min and washed in PBS. Cells were incubated with 0.1% Triton X-100 (ST795, Beyotime) at RT for 0.5 h and then treated with goat serum at RT for 15 min. Samples were cultured with primary antibody including NF- κ B p65 (dilution 1:100 in PBS, A2547, abclonal) and NLRP3 (dilution 1:100 in PBS, A12694, abclonal) overnight at 4 °C and incubated with Cy3-labeled goat anti-rabbit IgG (dilution 1:200 in PBS, A0516, Beyotime) in the dark at RT for 1 h. After being washed with PBS, cells were stained with DAPI (D106471, Aladdin, China) and visualized with a microscope.

Western blotting

Synovium tissues and treated cells were lysed in RIPA lysate (R0010, Solarbio) containing 1 mM PMSF (P0010, Solarbio) on ice for 5 min, respectively. After centrifugation (4 °C, 10,000 g, 5 min), the supernatant was used as total protein extraction. Nuclear protein was extracted from tissues or cells with the kit (R0050, Solarbio) in accordance with the manufacturer's protocol. The protein concentration was quantified by BCA kit (PC0020, Solarbio). 20 μ g of

protein were separated on 5, 8, 10, and 13% gels with 5% concentration gel. After electrophoresis, the separated proteins were transferred onto PVDF membranes (IPVH00010, Millipore, USA). The membranes were blocked with 5% skimmed milk diluted in Tris-buffered saline with Tween 20 (TBST) at RT for 60 min. Then the membranes were incubated with primary antibody at 4 °C overnight and cultured with secondary antibody (37 °C, 1 h). Protein bands were observed by the gel imaging system (WD9134B, Beijing Liuyi, China) using ECL kit (PE0010, Solarbio). The information of antibodies utilized in this study was as follows: GAPDH (1:10,000, 60004-1-Ig, proteintech, China); Histone H3 (1:5000, GTX122148, Gene Tex, USA); NLRP3 (1:1000, A12694), ASC (1:1000, A1170), IL-1 β (1:1000, A11369), TLR4 (1:1000, A5258), MyD88 (1:1000, A0980), and NF- κ B p65 (1:1000, A19653) purchased from abclonal; caspase-1 (1:500, WL03450, wanleibio, China); p-IKK β (Ser181, 1:1000, AF3013), IKK β (1:1000, AF6009), and p-NF- κ B p65 (Ser536, 1:1000, AF2006) provided by Affinity (China); goat anti-rabbit IgG-HRP (1:3000, SE134) and goat anti-mouse IgG-HRP (1:3000, SE131) supplied by Solarbio.

Electrophoretic mobility shift assay (EMSA)

Nuclear protein was extracted from synovium tissues via the kit (R0050, Solarbio) in the light of the manufacturer's instructions. The protein concentration was quantified by BCA kit (PC0020, Solarbio). EMSA was performed using the kit (BITF282, Viagene, China). Briefly, the nuclear extracts were incubated with biotin-labeled NF- κ B p65 probes and reacted for 20 min at RT. The reaction mixtures were separated by 6.5% non-denaturing PAGE and transferred on the membranes. The membranes were DNA cross-linked, incubated with streptavidin-HRP for 20 min at RT, and visualized with ECL kit.

Statistical analysis

Results were expressed as means \pm SD. For comparing between multigroups, one-way ANOVA followed by post hoc Tukey's test was carried out, except the statistical analysis of right palm swelling index (two-way ANOVA). $P < 0.05$ was considered statistically significant.

Results

Dioscin alleviated the severity of GA in mice

In this study, a GA mouse model was established. Compared to control mice, MSU stimulation increased the right palm swelling (Fig. 1a). By contrast, dioscin

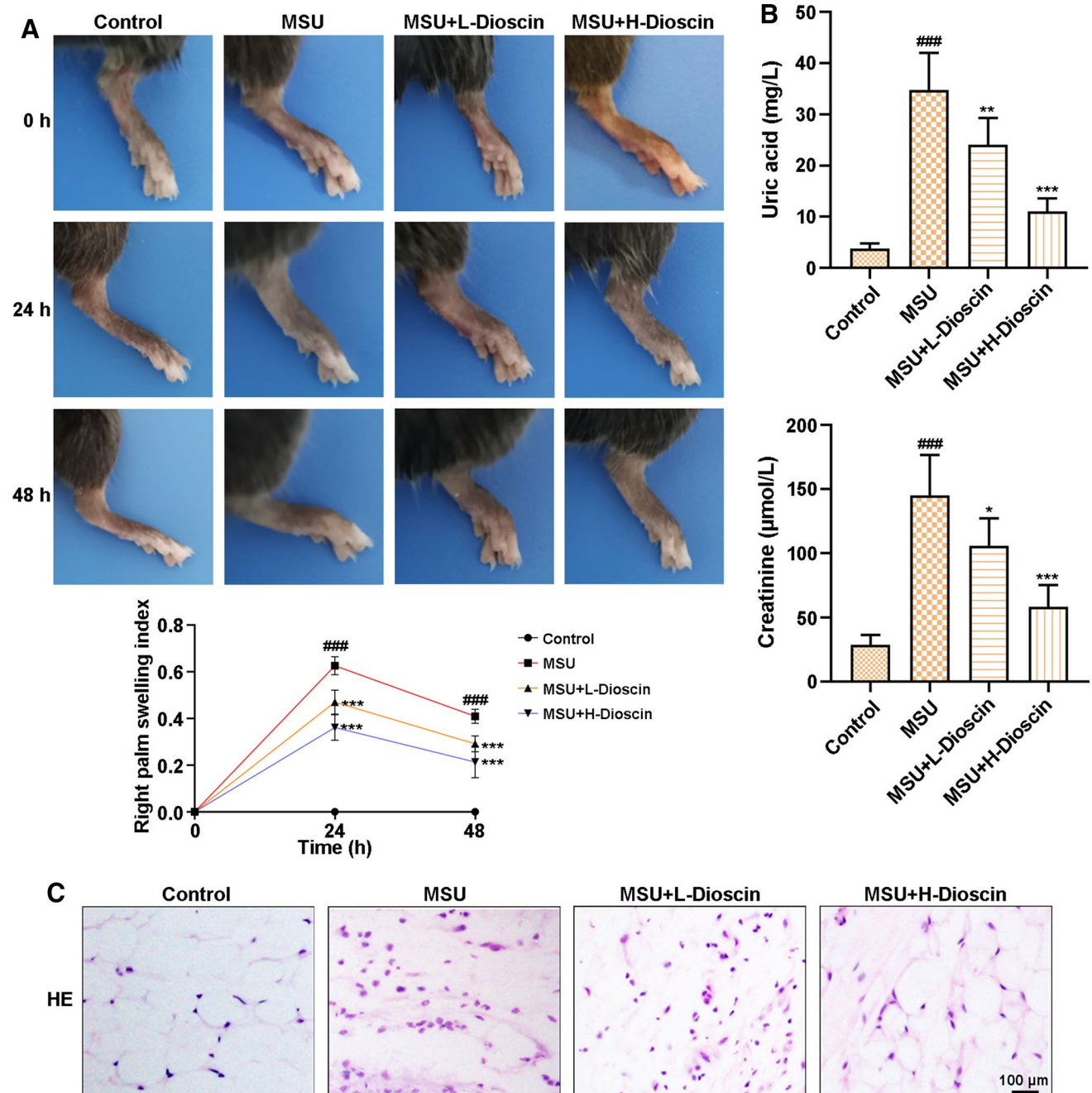


Fig. 1 Dioscin alleviated the severity of gouty arthritis in mice. Mice were administrated by dioscin (50 or 100 mg/kg) by gavage every day, and/or MSU (25 mg/mL) was injected into the right ankle of the mouse on the 8th day after intragastric administration for 1 h. **a** Swollen right palm in mice was photographed at 0, 24, and 48 h. Meanwhile, the degree of right palm swelling was evaluated. **b** The levels

of uric acid and creatinine in blood were detected with corresponding kits. Results were expressed as means \pm SD ($N=6$). $^{###}P < 0.001$ compared with control group; $^*P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with MSU group. **c** The mouse synovium tissues were stained with H&E and observed under a microscope. Scale bar: 100 μ m. MSU monosodium urate, H&E hematoxylin–eosin

administration prominently improved the swelling in MSU-treated mice. Additionally, dioscin evidently lowered the increase in uric acid and creatinine contents induced by MSU (Fig. 1b). H&E staining indicated that dioscin could attenuate inflammatory symptoms in

MSU-mediated GA via decreased neutrophil infiltration (Fig. 1c). Based on the above findings, dioscin alleviated the severity of GA in mice.

Dioscin repressed inflammasome NLRP3 activation in mouse synovium tissues

MSU enhanced the levels of IL-1 β as well as other pro-inflammatory factors including IL-6 and TNF- α in mouse synovium tissues (Fig. 2a). However, administration of dioscin significantly reversed these levels. Furthermore, the expression of NLRP3 was measured with immunohistochemistry staining. As shown in Fig. 2b, MSU obviously

elevated NLRP3 level when compared with control group, but dioscin lowered the level of NLRP3. To further demonstrate whether inflammasome NLRP3 was activated, we detected some protein expressions utilizing western blotting. Figure 2c indicated that dioscin reduced MSU-induced NLRP3, ASC, cleaved-caspase-1, and IL-1 β protein expressions. In addition, there were no remarkable changes in the levels of caspase-1 and pro-IL-1 β in mice

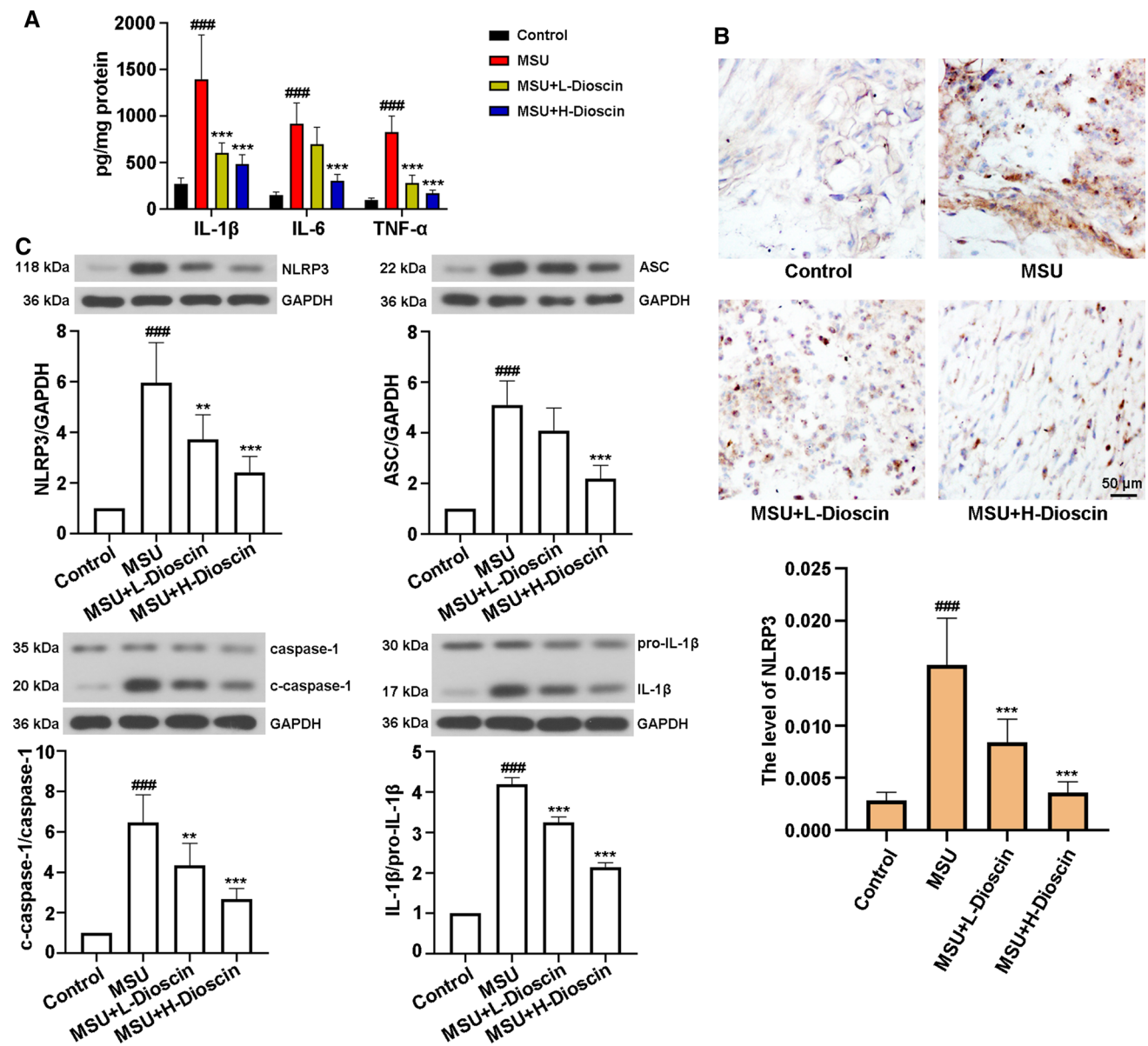


Fig. 2 Dioscin repressed NLRP3 activation in mouse synovium tissues. **a** IL-1 β , IL-6, and TNF- α levels were measured in synovium tissues using ELISA. **b** The expression of NLRP3 in synovium tissues was assessed via immunohistochemistry assay. Scale bar: 50 μ m. **c** NLRP3, ASC, caspase-1, c-caspase-1, pro-IL-1 β , and IL-1 β protein expressions in synovium tissues were detected with western blotting. GAPDH was utilized as internal reference. Data were presented

as means \pm SD ($N=6$). ### $P < 0.001$ compared with control group; ** $P < 0.01$ and *** $P < 0.001$ compared with MSU group. NLRP3 NLR family pyrin domain containing 3, IL-1 β interleukin-1 β , IL-6 interleukin-6, TNF- α tumor necrosis factor- α , ELISA enzyme-linked immunosorbent assay, ASC apoptosis-associated speck-like protein containing a caspase recruitment domain, c-caspase-1 cleaved-caspase-1

with and without treatment. The above results revealed that dioscin could inhibit the activation of NLRP3.

Dioscin inhibited the activation of TLR4/NF- κ B pathway in mouse synovium tissues

We found that the expressions of TLR4, MyD88, p-IKK β , p-p65, and NF- κ B p65 in nuclei were increased in mice treated with MSU (Fig. 3a). In contrast, dioscin obviously decreased the increase of these protein levels induced by

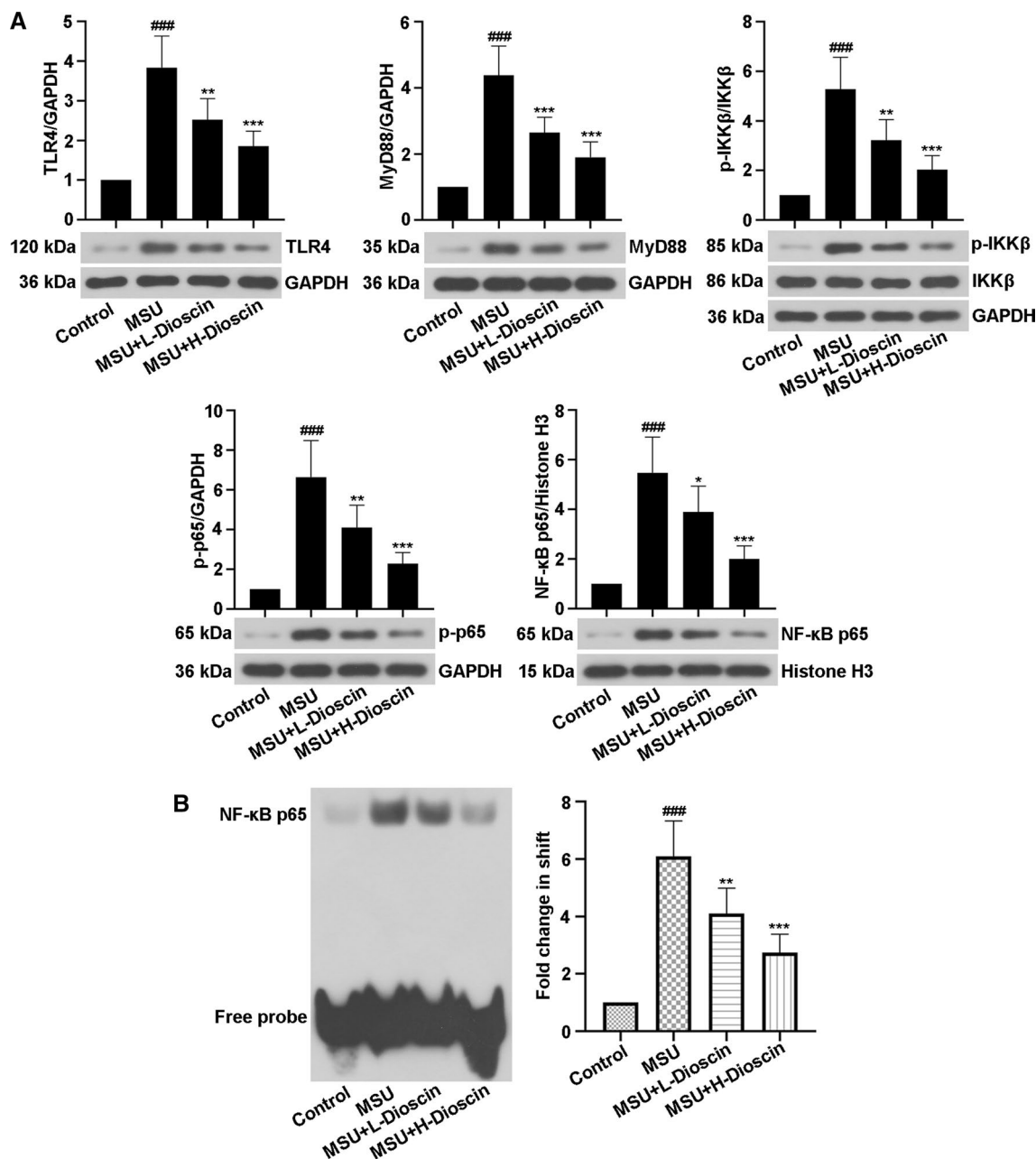


Fig. 3 Dioscin inhibited the activation of TLR4/NF- κ B pathway in mouse synovium tissues. **a** The levels of TLR4, MyD88, p-IKK β , IKK β , p-p65 in cytoplasm, and NF- κ B p65 in nuclei were assessed in synovium tissues by western blotting and quantified. GAPDH and Histone H3 were acted as internal references, respectively. **b** The NF- κ B p65-DNA binding activity in synovium tissues were ana-

lyzed by EMSA with a biotin-labeled probe. Results were expressed as means \pm SD ($N=6$). ### $P < 0.001$ compared with control group; ** $P < 0.01$ and *** $P < 0.001$ compared with MSU group. TLR4 toll-like receptor 4, NF- κ B nuclear factor- κ B, MyD88 myeloid differentiation primary response gene 88, p-IKK β phosphorylated IKK β , EMSA electrophoretic mobility shift assay

MSU. Moreover, the activation of NF- κ B signaling pathway was further evidenced by the elevated NF- κ B p65-DNA binding activity. As depicted in Fig. 3b, EMSA analysis suggested that the increase of NF- κ B p65-DNA activity mediated by MSU was inhibited by dioscin. These findings indicated that dioscin suppressed TLR4/NF- κ B pathway activation in MSU-treated mice.

Dioscin reduced inflammation in MSU-treated HS

In addition to in vivo assays, we investigated the anti-inflammatory effect of dioscin on MSU-mediated inflammation through in vitro experiments. It can be seen from Fig. 4a that cell viability was increased by MSU compared to unstimulated cells. Inversely, dioscin in the presence of MSU led to a reduction in cell viability. Figure 4b showed that IL-1 β , IL-6, as well as TNF- α levels were markedly lowered by dioscin. Besides, dioscin inhibited the nuclear translocation of NF- κ B p65 in MSU-treated cells (Fig. 4c). Then the protein expressions of mediators in TLR4/NF- κ B

signaling pathway were assessed with western blotting. As displayed in Fig. 4d, dioscin significantly decreased MSU-induced up-regulation of TLR4, MyD88, p-IKK β , p-p65, and NF- κ B p65 in nuclei levels. Considering these results, dioscin effectively reduced inflammation possibly via inhibition of MSU-induced activation of TLR4/NF- κ B signaling pathway in cells.

Dioscin suppressed NLRP3 activation in HS induced by MSU

ELISA results indicated that dioscin repressed MSU-induced IL-1 β generation (Fig. 5a). Similarly, CLI-095 (TLR4 inhibitor) also lowered the level of IL-1 β . Figure 5b revealed that the level of NLRP3 was increased after stimulation with MSU, however, dioscin noticeably reversed this process. Apart from these, the protein expressions of NLRP3 and its downstream proteins were evaluated. As shown in Fig. 5c, NLRP3, ASC, cleaved-caspase-1, and IL-1 β protein expressions were evidently down-regulated by dioscin

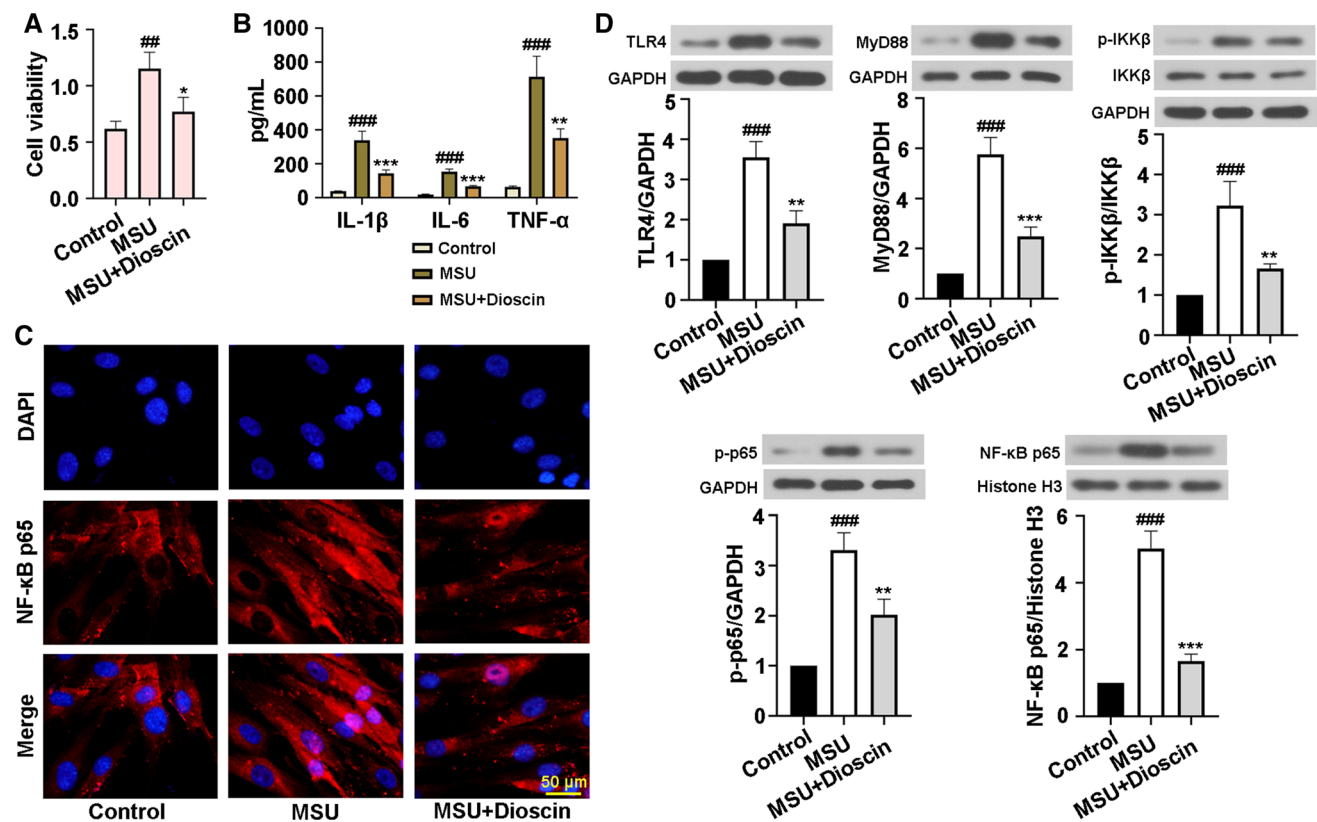


Fig. 4 Dioscin reduced inflammation in MSU-treated primary human synoviocytes (HS). Cells were administrated by dioscin (15 ng/mL) for 24 h, and then treated with MSU (100 μ g/mL) for 12 h. **a** After incubation, cell viability was detected via MTT assay. **b** The levels of IL-1 β , IL-6, and TNF- α in cell supernatant were assessed by ELISA. **c** The level of NF- κ B p65 in HS was evaluated with immunofluorescence assay. Scale bar: 50 μ m. **d** The protein expressions of TLR4,

MyD88, p-IKK β , IKK β , p-p65 in cytoplasm, and NF- κ B p65 in nuclei were measured utilizing western blotting. GAPDH and Histone H3 were acted as internal references, respectively. Data were presented as means \pm SD ($N=3$). ### $P<0.001$ and #### $P<0.0001$ compared with control group; * $P<0.05$, ** $P<0.01$, and *** $P<0.001$ compared with MSU group

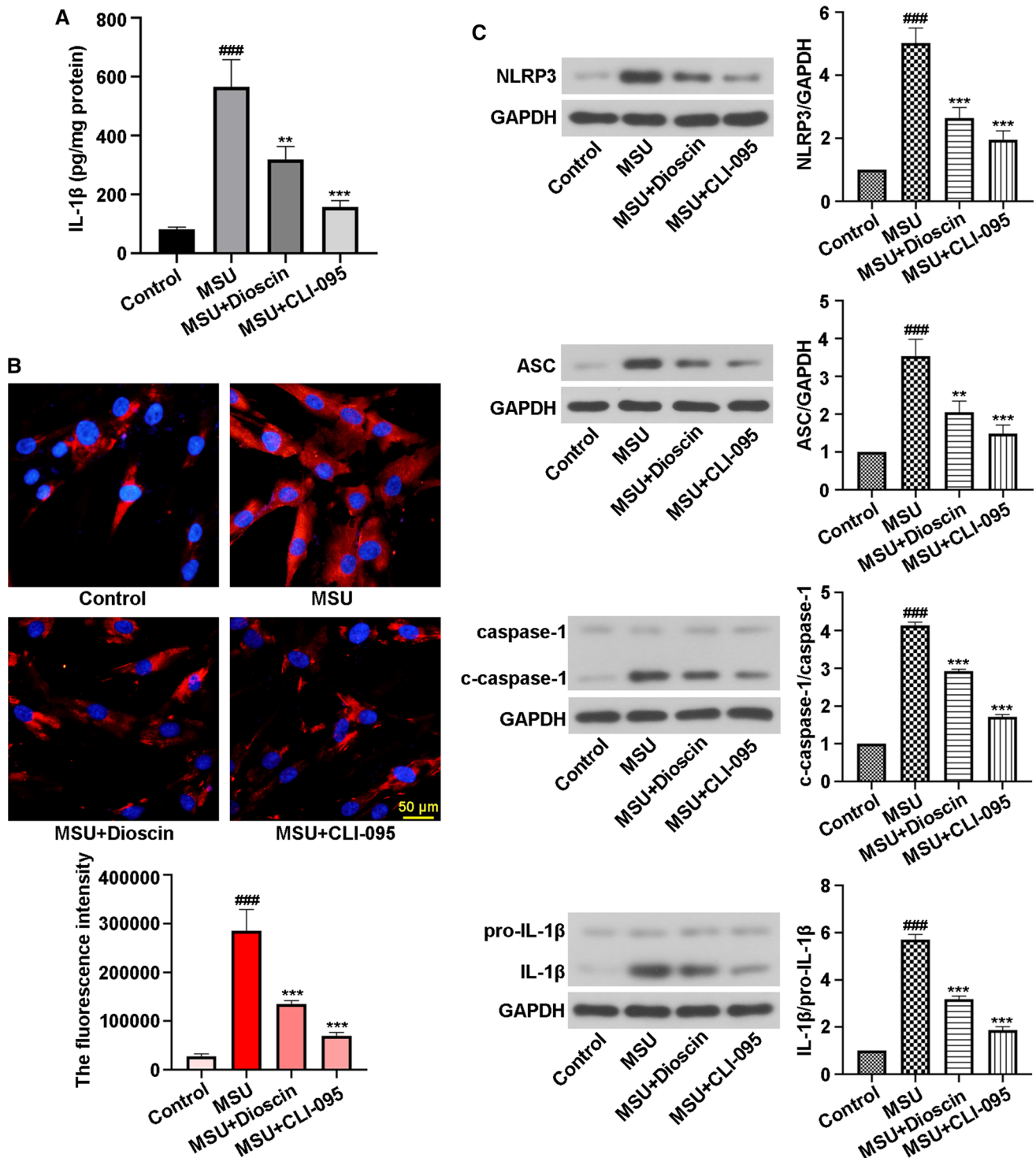


Fig. 5 Dioscin suppressed NLRP3 activation in HS induced by MSU. Cells were treated with dioscin (15 ng/mL) or TLR4 inhibitor (CLI-095(TAK-242), 5 μ mol/L) for 24 h, and then administrated by MSU (100 μ g/mL) for 12 h. **a** After incubation, the level of IL-1 β was determined with ELISA. **b** The expression of NLRP3 was detected by immunofluorescence assay. Scale bar: 50 μ m. **c** The protein expres-

sions of NLRP3, ASC, caspase-1, c-caspase-1, pro-IL-1 β , and IL-1 β were assessed via western blotting. GAPDH was used as internal reference. Results were expressed as means \pm SD ($N=3$). ^{###} $P < 0.001$ compared with control group; ^{**} $P < 0.01$ and ^{***} $P < 0.001$ compared with MSU group

in MSU-treated cells. Meanwhile, no obvious alternations in caspase-1 and pro-IL-1 β levels were seen in cells with and without treatment. The above findings suggested that dioscin suppressed MSU-induced NLRP3 activation.

Discussion

Emerging studies paid attention to investigating the mechanism of pain and inflammatory response of GA [12]. This study showed that the mouse right palm swelling was serious after MSU injection. At the same time, the levels of uric acid and creatinine were obviously elevated in the presence of MSU. Histopathological results demonstrated evidence for the inflammation in synovial tissues. It provided an animal model of GA for further studying the underlying mechanisms. Our researches confirmed that dioscin could attenuate MSU-induced GA in mice through lowered uric acid as well as creatinine levels and decreasing neutrophil infiltration.

MSU-mediated inflammatory reaction is specially triggered via the activation of inflammasome within monocytes/macrophages, leading to the release of inflammatory markers within the joint [15]. IL-1 β is considered as one of the important pro-inflammatory mediators in GA induced by MSU [3]. In the present study, it was found that the level of IL-1 β was obviously increased in model group. We also determined other indicators (IL-6 and TNF- α) elevated in MSU-treated mice and cells. On the contrary, dioscin decreased these levels. Similar findings were observed in a previous study [16]. NLRP3 inflammasome has been reported to play an essential part in the cellular defense against invading pathogens [17]. Furthermore, as evidenced by Marchetti et al. [18], in murine models of acute arthritis, NLRP3 inhibitor OLT1177 could effectively repress the release of IL-1 β as well as IL-18 and subsequently mitigate the process of GA. It has been documented that MSU induces the activation of NLRP3, and then an apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and NLRP3 are gathered, which has an interaction with caspase-1 [19]. This process promotes the cleavage of caspase-1. Also, it is well recognized that the release of IL-1 β can be regulated via caspase-1-containing inflammasomes such as NLRP3 [17]. Herein, the protein expressions of NLRP3, ASC, cleaved-caspase-1, and IL-1 β were up-regulated by stimulation with MSU in vivo and in vitro assays. Nevertheless, these levels were reversed by dioscin, which was similar to the previous researches [19, 20].

Recently, TLRs, especially TLR4, have been acted as a family of vital proteins participating in immune response [21]. Zhou et al. [22] reported that TLR4 could be recognized as the receptor of endogenous MSU. IL-1 β , IL-6, and TNF- α are very crucial components of the cascade

downstream of TLRs [23]. The increased levels of these cytokines can further facilitate the production of a set of adherence factors expressed in endothelial cells to recruit more neutrophils accumulated in urate deposition site. Activated neutrophils are capable of exacerbating the inflammation through inducing autophagy and conversely secreting the majority of inflammatory mediators such as IL-1 β [24]. Many studies revealed that MSU could directly bind to some cell membrane proteins (TLR2 and TLR4), thus activating NF- κ B via up-regulating the protein level of myeloid differentiation factor 88 (MyD88) [25]. Besides, a previous research indicated that curcumin attenuated MSU-mediated inflammation through inhibition of TLR4 pathway activation with in vivo and in vitro experiments [20]. In this study, MSU-induced elevated protein expressions of TLR4 and MyD88 were inhibited by dioscin, demonstrating that dioscin probably suppressed the activation of TLR4 signaling pathway. The above results were consistent with Chen's study [26] that miR-146a alleviated joint inflammation of acute arthritis rats by inhibition of TLR4 pathway.

In addition to TLR4 pathway, NF- κ B signaling pathway is the key indicator in inflammatory reaction and consists of a family of transcription factors. Accumulating literatures reported that the activation of NF- κ B pathway could induce the generation of inflammatory cytokines which resulted in the articular joint injury [14]. Under normal physiological conditions, NF- κ B existed in the cytoplasm in an inactive form, the heterodimer of p65 and p50 subunits, which binds to I κ B α protein [27]. Protein phosphorylation is an extensively modulatory way in the body, which is a critical event in cell signal transduction [12]. Once inflammatory response occurs, IKK β controls NF- κ B signaling pathway activation via phosphorylation and subsequent degradation of I κ B α [28]. Then released NF- κ B p65 is translocated into nucleus in which it binds to specific DNA sequences and causes transcription. As a result, a series of inflammatory indicators will be generated and GA happens. A report previously described was that morin, a bioflavonoid, inhibited MSU-induced inflammation in RAW264.7 cells through inhibition of inflammatory cytokine production and NF- κ B signaling pathway activation [29]. In the current study, the levels of p-IKK β , p-p65, and NF- κ B p65 in nuclei were enhanced and the NF- κ B p65-DNA activity was increased after stimulation with MSU. By contrast, dioscin reversed these changes via in vivo and in vitro assays, demonstrating that dioscin possibly inhibited the activation of NF- κ B signaling pathway. Similar results were found in the earlier works previously reported [26, 30].

In conclusion, dioscin had a protective effect against MSU-induced inflammation through in vivo and in vitro experiments. The potential molecular mechanism was possibly accomplished by reducing the production of pro-inflammatory cytokines and inhibiting TLR4/NF- κ B signaling

pathway activation. Hence, dioscin could be a novel drug for the treatment of GA in future.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest in this article.

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