

Triptolide Inhibits the Activation of NLRP3 Inflammasome by Inhibiting NF- κ B Pathway and Improves Myocardial Fibrosis

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Research

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Abstract

Objective

Triptolide (TPL) is identified to be involved in the treatment for myocardial fibrosis (MF). This study investigated the mechanism of TPL in MF in rats and observed its effect on NLRP3 inflammasome signaling pathway.

Materials and Methods

The MF rat model was established by subcutaneous injection of isoproterenol (ISO), and treated by subcutaneous injection of TPL. After modeling for 1 week, the cardiac function of rats was evaluated, including LVEF, LVFS, LVES, LVED LVIDs and LVPWs. The HMI and LVMI were measured. The expressions of ANP, BNP, inflammatory related factors (IL-1 β , IL-18, TNF- α , MCP-1, VCAM-1), and NLRP3 inflammasome factors (NLRP3, ASC, caspase-1) in rats were detected. HE staining and Masson staining were used to observe myocardial cell inflammation and fibrosis of rats.

Results

LVED, LVES, LVIDs and LVPWs of MF group were significantly upregulated, LVEF and LVFS were significantly downregulated, HMI and LVMI were upregulated, while TPL treatment reversed these trends; TPL treatment downregulated the tissue injury and improved the pathological damage of MF rats. TPL treatment downregulated the levels of inflammatory factors and fibrosis factors, and inhibited the activation of NLRP3 inflammasome. Activation of NLRP3 inflammasome or NF- κ B pathway reversed the effect of TPL on MF.

Conclusions

TPL inhibited the activation of NLRP3 inflammasome by inhibiting NF- κ B pathway, and improved the degree of MF in MF rats.

Introduction

Myocardial fibrosis (MF) is defined as various quantitative and qualitative changes of the myocardial interstitial collagen network which occurs in response of cardiac ischemic injuries, systemic diseases, drugs, or any other harmful stimulation affecting the circulatory system or the heart itself [1]. MF is a common phenomenon in the late stage of many heart diseases and is a predictor of sudden cardiac death [2], which may be caused by a series of pathological processes [3]. Myocardial fibroblasts become pro-inflammatory cells and activating the inflammasome in the early stages of infarct healing [4]. Evidence also supports the effect of inflammation on the initiation and promotion of diverse cardiovascular diseases, including MF [5]. MF changes the myocardium architecture, leads to cardiac dysfunction, arrhythmias and ischemia, and thus influences the clinical course and prognosis of patients

with heart failure [6]. However, there still lacks satisfactory treatment for MF and further work is needed for the effective intervention of MF.

Triptolide (TPL) is a main active component extracted from traditional Chinese herbal medicine *Tripterygium wilfordii*, which exhibits multiple pharmacological effects [7], including potent antitumor, immunosuppressive, and anti-inflammatory properties, but the effects are limited in clinic because of the poor solubility, toxicity and bioavailability [8]. The value of TPL in the treatment of various inflammatory disorders and cancers has been emphasized over the past few years [9, 10]. Evidence has also shown that the TPL plays a role in attenuating cardiac fibrosis [11]. Inflammation is one of the best-studied mediators related to cardiac fibrosis [12]. The NLRP3 inflammasome is noticed as a key to regulate metabolic inflammation [13]. The inhibitor of NLRP3 can alleviate the MF [14]. It is reported that an appropriate dose of TPL can inhibit the activation of NLRP3 inflammasome [15]. It is shown that TPL can downregulate NLRP3 [16]. Collectively, TPL may play an effective role in interrupting the activation of NLRP3 inflammasome to improve MF.

At present, there are few reports on the effect of TPL on treating MF induced by isoproterenol (ISO), and the mechanism of TPL improving the condition of MF needs to be further explored. This study investigated the protective mechanism of TPL on MF rats, and shall provide a new theoretical basis for the improvement of TPL on MF.

Materials And Methods

Ethics statement

All procedures were authorized by the academic ethics committee of The Central Hospital Affiliated to Shaoxing University (ethics committee approved number: 2019-003). All procedures were strictly implemented by the guidelines of Animal Care Committee of The Central Hospital Affiliated to Shaoxing University. All the laboratory procedures were used to reduce the pain of the rats, such as heating pads, disinfection and replenishing fluids with saline.

Laboratory animals and treatment

Adult male Sprague-Dawley (SD) rats, 8–10 weeks old, weighing 220–270 g, were purchased from Shanghai Shrek experimental animal Co., Ltd (Shanghai, China). The rats were raised in a clean room in a 12-h light/dark cycle at 20°C with 50% and 60% humidity. All animals were euthanized by intraperitoneal injection of pentobarbital sodium ≥ 100 mg/kg.

All the 42 SD rats were randomly assigned into 7 equal groups, with 4 groups for studying the effect of TPL on MF: sham group (subcutaneously injected with 5 mg/kg/d saline), myocardial fibrosis (MF) group (subcutaneously injected with 5 mg/kg/d isoproterenol, ISO), dimethyl sulfoxide (DMSO) group (subcutaneously injected with ISO at 5 mg/kg/d and DMSO at 200 μ g/kg/d) and triptolide (TPL) group (subcutaneously injected with ISO at 5 mg/kg/d and intraperitoneal injected with TPL at 200 μ g/kg/d,

which was dissolved in DMSO) (Sigma, St Louis, MO, USA) for 7 d [17]. The heart function was measured 7 d after injection and following experiments were carried out. The other 3 groups were used for the joint experiment to study the effect of TPL on MF through NLRP3 inflammasome and NF- κ B: TPL + PBS group, TPL + Nigericin group and TPL + Jaceosidin group. The rats in TPL + Nigericin and TPL + Jaceosidin groups were intraperitoneally injected with NLRP3 inflammasome activator Nigericin (2.5 mg/kg/d) (Sigma) or NF- κ B pathway activator Jaceosidin (2.5 mg/kg/d) (Sigma) for 7 d [18, 19], and the injection of PBS was used as the control group.

Cardiac function test

After 7 d of continuous injection, echocardiography was performed to evaluate cardiac function. The rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (40 mg/kg) and fixed on the examination table in supine position. The chest hair was removed and the electrocardiogram (ECG) was recorded simultaneously. The left ventricular end systolic diameter (LVES), left ventricular end diastolic diameter (LVED), left ventricular diameters (LVIDs) and left ventricular systolic posterior wall thickness (LVPWs) were measured by M-mode echocardiography images in parasternal long axis view of left ventricle. The system calculated the left ventricular ejection fraction (LVEF) and left indoor fractional shortening (LVFS) automatically. Vevo 2100 small animal ultrasonograph (Fujifilm VisualSonics, Inc., Toronto, Canada) was used for analysis.

Measurement of heart mass index (HMI) and left ventricular mass index (LVMI)

All animals were euthanized by an intraperitoneal injection of pentobarbital sodium \geq 100 mg/kg after cardiac function test [20]. The hearts of rats were collected and the large blood vessels and adipose tissues were removed. The hearts were washed with PBS for 3 times, dried with filter paper, and weighed. The left ventricle was separated and weighed. HMI was calculated by heart weight/rat body weight, and LVMI was calculated by left ventricular weight/rat body weight.

Sample collection

All animals were euthanized by an intraperitoneal injection of pentobarbital sodium \geq 100 mg/kg after cardiac function test [20]. The myocardial tissues of some rats were taken out and embedded in paraffin and each specimen was cut into sections at 4 μ m for histological experiments. The myocardial tissues of the rest rats were made into homogenate for subsequent detection.

Hematoxylin and eosin (HE) staining

The rat hearts were soaked in 4% paraformaldehyde for 24 h, and each specimen was cut into sections at 4 μ m. HE staining was performed after dewaxing. The sections were stained with hematoxylin for 5 min and stained with eosin for 5 s. Then sections were then dehydrated, cleared, and sealed by neutral resin and finally the pathological damage of rats was observed by microscope.

Masson staining

The prepared myocardial tissue sections were treated with Masson staining solution (Sigma), 1% phosphotungstic acid solution, aniline blue solution, and 1% glacial acetic acid solution successively, and images were acquired and analyzed after dehydration and clearance. The collagen fibers were stained blue, the nucleus was stained blue, and the cytoplasm was stained red. Blind method was used to analyze 5 visual fields in each section and the blue area of collagen fibers. The collagen volume fraction (CVF) was measured. $CVF = \frac{\text{the blue area of collagen fibers}}{\text{full field area}} \times 100\%$, and the mean value was calculate.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA of myocardial tissue homogenate was respectively extracted by RNAiso Plus (TAKARA, Otsu, Shiga, Japan) and Trizol LS Regent (TAKARA). The reliability of extracted RNA was verified by formaldehyde denaturation electrophoresis. Subsequently, the reverse transcription experiment was performed with PrimeScript™ RT kit (TAKARA) in strict accordance with the instructions. SYBR Premix Ex Taq (TAKARA) was used to quantify the relative expression of genes by RT-qPCR. GAPDH was used as reference gene. The primers in each group were shown in Table 1.

Table 1
Primer sequence

Gene	Forward	Reverse
ANP	TTCTTCCTCGTCTTGGCCTTT	GACCTCATCTTCTACCGGCATCT
BNP	CACCGCTGGGAGGTCACT	GTGAGGCCTTGGTCCTTCAA
IL-1 β	CCCTGCAGCTGGAGAGTGTGG	ACGTGAGCAAGATCTTCCCA
IL-18	ACGTGAGCAAGATCTTCCCA	CCCAGAAGTCATCGTCAGTG
TNF- α	TGAAGGACAAACCGAAGG	GGAAGAGCAGAAAGCGATA
MCP-1	CAGATCTCTCTTCCCTCCACCACTAT	CAGGCAGCAACTGTGAACAAC
VCAM-1	CTCAGGTGGCTGCACAAGTT	AGAGCTCAACACAAGCGTGG
NLRP3	GTTTGACCCCGATGATGAGC	CTTGTGGATGGGTGGGTTTG
ASC	TGAAGGACAAACCGAAGG	GGAAGAGCAGAAAGCGATA
TGF- β 1	CCCTGCAGCTGGAGAGTGTGG	TGTGCTCTGCTTGAGAGGTGCT
COL1	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
COL3	CTGTAACATGGAACTGGGGAAA	CCATAGCTGAACTGAAAACCACC
GAPDH	AAGAAGGTGGTGAAGCAGGC	TCCACCACCCAGTTGCTGTA

Western blot (WB) analysis

Radio-immunoprecipitation assay (RIPA) lysate containing protease inhibitor (Beyotime, Shanghai, China) was used to lyse cells to extract protein. Protein concentration was determined by a bicinchoninic acid (BCA) kit (Biyuntian). The protein was denatured at 100°C for 5 min, separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA, USA). The membranes were incubated with buffer at 4°C for 2 h, and added with rabbit anti-mouse primary antibodies NLRP3 (1:500, ab270449, Abcam), ASC (1:1000, ab47092, Abcam), p-P65 (1:1000, ab194726, Abcam), and Caspase-1 (1:1000, #2225, CST) at 4°C overnight. After three times of cleaning of PVDF membranes with tris buffered saline and Tween-20 (TBST), the PVDF membranes were incubated with anti-rabbit IgG secondary antibody (1:2000, ab6721, Abcam) 4°C for 4 h. The bands were visualized using chemiluminescence and analyzed by gray analysis, and GAPDH was the control of total protein content.

Statistical analysis

SPSS21.0 statistical software (IBM Corp. Armonk, NY, USA) was used to analyze data. Kolmogorov-Smirnov test showed that the data were in normal distribution and expressed as mean \pm standard deviation. One-way analysis of variance (ANOVA) was used for comparison among multi-groups. Tukey's multiple comparisons test was used for post hoc test. *P* value was obtained by a bilateral test, where $p < 0.05$ indicated statistical significance and $p < 0.01$ indicated a very significant difference.

Results

TPL improved cardiac dysfunction in MF rats

TPL is the main active component of tripterygium wilfordii hook, which has been reported for many times that for the functions of anti-inflammation, anti-tumor and immunosuppression [9]. However, the role of TPL in MF rats remains to be further studied. One week after ISO induction, the cardiac function of rats was measured by small animal ultrasonograph. Compared with sham-operated rats, LVED, LVES, LVIDs and LVPWs of rats in MF group and DMSO group were significantly upregulated, while LVEF (%) and LVFS (%) were significantly decreased, which indicated that the systolic and diastolic functions of rat hearts induced by ISO were significantly impaired, and cardiac dysfunction existed, while TPL treatment effectively improved the cardiac dysfunction of MF rats (all $p < 0.05$, Fig. 1A-F). The rat heart tissues were collected. Compared with sham group, the HMI, and especially the LVMI in MF group and DMSO group were significantly increased, while TPL treatment significantly improved the increase of HMI and LVMI induced by ISO (Fig. 1G). Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are secreted by ventricular myocytes, and their concentrations are closely related to cardiac function [21, 9]. Therefore, the mRNA expressions of ANP and BNP in rat myocardium were measured. The results showed that the levels of ANP and BNP in MF group and DMSO group were higher than those in sham group, while the levels of ANP and BNP in TPL group were significantly decreased (all $p < 0.01$, Fig. 1H). These results suggested that TPL could effectively improve cardiac dysfunction in MF rats.

TPL reduced the degree of fibrosis in rats

MF could cause myocardial tissue disorder and large amount of collagen deposition [22]. Therefore, HE staining and Masson staining were used to observe the pathological morphology and MF of rats in each group. HE staining showed that compared with sham group, the disorder of myocardial tissue structure and injury of MF group and DMSO group were significantly aggravated, while the tissue injury degree of TPL group was effectively decreased ($p < 0.01$), which indicated that TPL treatment could improve degree of the pathological damage of MF rats. Masson staining demonstrated that there were abundant collagen fibers in the rats of MF group and DMSO group (Fig. 2A). CVF quantitative analysis indicated that the collagen volume fraction of rats in MF group and DMSO group was significantly increased relative to that of sham group, and TPL treatment significantly reduced CVF of rats ($p < 0.01$, Fig. 2B). RT-qPCR was used to detect the expression of fibrosis related factors. Expressions of fibrosis related factors TGF- β 1, COL1 and COL3 in MF group and DMSO group were higher than that in sham group, while those in TPL group were lower than that in MF group (all $p < 0.01$, Fig. 2C). These results suggested that TPL could downregulate the degree of fibrosis in rats.

TPL downregulated the level of inflammatory factors in MF rats

Cardiac dysfunction was often accompanied by inflammation [23, 24]. The mRNA and protein levels of inflammatory factors IL-1 β , IL-18 and TNF- α in myocardial tissue of rats were detected in each group. Compared with sham group, the mRNA levels of inflammatory factors IL-1 β , IL-18 and TNF- α in MF group and DMSO group were significantly upregulated, while TPL treatment effectively decreased the expression of inflammatory factors (all $p < 0.01$, Fig. 3A). Monocyte chemoattractant protein 1 (MCP-1) and vascular cell adhesion molecule 1 (VCAM-1) are also key inflammatory factors, which could promote the occurrence of inflammatory reaction [25, 26]. The mRNA expressions of MCP-1 and VCAM-1 were also detected, and the results were consistent with our predicted trend. TPL treatment could downregulate the expression of MCP-1 and VCAM-1 in MF rats (all $p < 0.01$, Fig. 3B). These results suggested that TPL could downregulate the inflammatory level of MF rats.

TPL inhibited the activation of NLRP3 inflammasome in MF rats

Subsequently, RT-qPCR and WB were used to respectively detect the main components of NLRP3 inflammasome mRNA and protein levels of NLRP3 and ASC. Compared with sham group, the mRNA and protein levels of NLRP3 and ASC in MF group and DMSO group were significantly upregulated, and TPL treatment effectively downregulated their expressions (all $p < 0.01$, Fig. 4A-D). NLRP3 inflammasome can induce pro-caspase-1 self-cleavage to activated caspase-1 [27]. Therefore, the activation of caspase-1 was detected by WB, and the results also showed that compared with sham group, the expression of caspase-1 in MF group and DMSO group were significantly upregulated, while TPL treatment effectively

downregulated the expression of caspase-1 (all $p < 0.05$, Fig. 4E). It was suggested that TPL inhibited the activation of NLRP3 inflammasome in MF rats.

TPL improved fibrosis degree of MF rats by inhibiting the activation of NLRP3 inflammasome pathway

It was confirmed through previous experiments that TPL could inhibit the inflammasome of NLRP3 and improve the MF. Therefore, we speculated that TPL might improve the MF by regulating the NLRP3 inflammatory pathway. In order to verify the conjecture, a joint experiment was conducted: NLRP3 inflammasome activator Nigericin was added in TPL-treated group, with the addition of PBS as control group. The changes of fibrosis degree in MF rats were observed. After adding Nigericin, LVED, LVES, LVIDs and LVPWs were significantly increased, LVEF and LVFS were significantly decreased (Fig. 5A-F), the myocardial tissue structure was disordered, the injury was aggravated, and the volume fraction of collagen was increased (Fig. 5G-H). Moreover, compared with TPL + PBS group, the mRNA expressions of inflammatory factors IL-1 β , IL-18 and TNF- α in TPL + Nigericin group were higher than those in control group (all $p < 0.01$, Fig. 5I), indicating that NLRP3 pathway was successfully interfered, and the inflammatory level of TPL + Nigericin group was upregulated. Subsequently, the mRNA expressions of fibrosis related factors (TGF- β 1, COL1 and COL3) were detected by RT-qPCR. The mRNA expressions of fibrosis related factors in TPL + Nigericin group were significantly higher than that in TPL + PBS group (all $p < 0.01$, Fig. 5J). These results suggested that TPL improved the degree of MF in MF rats by inhibiting the activation of NLRP3 inflammasome pathway.

TPL inhibited the activation of NLRP3 inflammasome by inhibiting NF- κ B pathway and thus improving the MF in rats

NLRP3 pathway is an important downstream pathway of NF- κ B pathway, and NF- κ B pathway is often reported to be associated with inflammation and MF [28, 29]. It was speculated that TPL might inhibit the activation of NLRP3 inflammasome by inhibiting the NF- κ B signaling pathway and improve the degree of MF. Therefore, the expression of NF- κ B pathway related proteins was detected, and it was found that P65 phosphorylation levels in MF group and DMSO group were higher than that in sham group, while TPL treatment effectively downregulated P65 phosphorylation level ($p < 0.01$, Fig. 6A). Subsequently, the NF- κ B pathway activator Jaceosidin was used to treat rats in the TPL group, and the injection of PBS was used as the control group. It was found that after Jaceosidin treatment, rat cardiac function indexes LVED, LVES, LVIDs and LVPWs were significantly increased, LVEF and LVFS were significantly decreased (Fig. 6B-G), the improvement of TPL on myocardial tissue structure damage was reversed, myocardial tissue damage was aggravated, and collagen volume fraction was increased (Fig. 6H-I). The changes of inflammatory and fibrosis related factors in myocardial tissue were detected by RT-qPCR. Levels of inflammatory factors and fibrosis factors in TPL + Jaceosidin group were higher than those in TPL + PBS group ($p < 0.01$, Fig. 6J-K). All the above results confirmed that TPL could improve the MF by inhibiting NF- κ B pathway and the activation of NLRP3 inflammasome.

Discussion

MF, mainly caused by the death of ischemic cells in the process of myocardial infarction that leads to a reparative response in which the damaged tissue is replaced with a fibrotic scar, can lead to impairment of cardiac function and finally causes adverse outcomes of heart failure [30]. However, there is no therapeutic strategy that can ensure its reversal at present [31]. Evidences have shown that TPL has the characteristics of anti-inflammatory and antitumor [9]. This study found that TPL could inhibit the activation of NLRP3 inflammasome and improve MF by inhibiting NF- κ B pathway.

This study found that TPL could inhibit the activation of NLRP3 inflammasome and improve MF by inhibiting NF- κ B pathway.

TPL has been shown to have potent antitumor activity in various preclinical cancer models [8], such as lung cancer, breast cancer and pancreatic cancer [32–34]. However, the effect of TPL on MF was still unclear. MF can contribute to cardiac dysfunction in many pathophysiologic conditions [12]. The levels of LVED, LVES, LVEF and LVFS are main indexes of cardiac function [35]. This study showed that TPL improved cardiac dysfunction in MF rats. LVED, LVES, LVIDs and LVPWs were significantly upregulated, while LVEF and LVFS were significantly decreased in MF rats, indicating that the systolic and diastolic functions of rat hearts were significantly impaired; and the cardiac dysfunction of MF rats was effectively improved by TPL treatment. The concentrations of ANP and BNP are closely associated with cardiac function [21]. The levels of ANP and BNP in MF rats were significantly higher, while the levels of ANP and BNP in TPL group were significantly decreased. Consistently, it has been revealed that TPL therapy improves cardiac function through upregulation of MAPK signaling transduction [36]. In brief, TPL could effectively improve cardiac dysfunction in MF rats.

MF can cause myocardial tissue disorder and collagen deposition [22]. The collagen fibers in the rats of MF and DMSO groups were abundant. The collagen volume fraction of rats in MF and DMSO groups was increased significantly, and TPL group reduced CVF of rats significantly. The tissue injury in MF rats was upregulated significantly while that in TPL group was decreased effectively, indicating that TPL could improve pathological damage of MF rats. TPL inhibits collagen gel and suppresses the production of fibronectin [37]. A study has shown an alleviation effect of TPL on the diabetic renal fibrosis by inhibiting miR-141-3p/PTEN/Akt/mTOR pathway [38]. This study explored the role of TPL in alleviating the fibrosis and the expression of fibrosis-related factors. The fibrosis related factors (TGF- β 1, COL1 and COL3) expressions in MF and DMSO groups were much higher, while those in TPL group were much lower. Consistently, TPL inhibits airway smooth muscle cells malignant behaviors induced by TGF- β 1 significantly [39]. These suggested that TPL could alleviate MF and downregulate the fibrosis related factors expression in MF rats.

Cardiac dysfunction is often accompanied by inflammation [24]. Our results showed that the levels of inflammatory factors (IL-1 β , IL-18 and TNF- α) in MF and DMSO groups were upregulated significantly, while TPL treatment decreased the expression of inflammatory factors effectively. It is reported that TPL can downregulate the expressions of IL-1 β and TNF- α [40]. TPL attenuates inflammatory responses in

endothelial cells [41]. In brief, these results suggested that TPL could downregulate the level of inflammatory in MF rats. NLRP3 inflammasome can finally produce a mature form of highly inflammatory cytokines [42]. The mRNA and protein levels of NLRP3 and ASC were increased significantly in MF and DMSO groups, while TPL treatment decreased their levels effectively. It is reported that TPL can inhibit the activation of NLRP3 inflammasome and protect against the inflammatory injury in diabetic kidney disease [15]. We speculate that TPL may improve MF by regulating NLRP3 inflammasome pathway. To test our hypothesis, we added NLRP3 inflammasome activator Nigericin in TPL group. Our results demonstrated that mRNA expressions of inflammatory factors and fibrosis related factors in TPL + Nigericin group were higher. There is a previous study supporting that TPL protects against pressure overload of hearts by inhibiting the activation of NLRP3 inflammasome [43]. Treatment with TPL exerts the anti-inflammatory effects by downregulating NLRP3 inflammasome in nephritis [44]. In brief, it was suggested that TPL improved MF by inhibiting the activation of NLRP3 inflammasome pathway.

NF- κ B pathway is reported to play an essential role in myocardial inflammation as a transcription factor [29]. TPL improves podocyte apoptosis and proteinuria by inhibiting NF- κ B/GADD45B pathway [45]. We deduced that TPL might inhibit NLRP3 inflammasome via inhibiting NF- κ B pathway. The results showed that P65 phosphorylation levels in MF and DMSO groups were elevated, while TPL treatment downregulated the levels effectively. Next, we treated the TPL group with NF- κ B pathway activator Jaceosidin. The levels of inflammatory factor and fibrosis factors in TPL + Jaceosidin group were significantly enhanced. TPL may suppress inflammatory response of endothelial cells by inhibiting activation of NF- κ B [41]. There is little research on the effect of TPL on inhibiting MF via NF- κ B pathway. Our study confirmed that TPL could improve MF by inhibiting NF- κ B pathway and the activation of NLRP3 inflammasome.

In summary, this study supported that TPL could inhibit the activation of NLRP3 inflammasome by inhibiting NF- κ B pathway, thus improving MF. Whether there is a deeper regulatory mechanism between TPL and NF- κ B pathway in MF remains to be further studied. Our results showed that TPL was only a preventive method and does not necessarily reverse the MF established after ISO injection. Its therapeutic effect needs to be further studied. Studies have shown that TPL can inhibit inflammation [46, 41]. There may be some feasibility in the TPL treatment. However, TPL is also the main toxic component, which has serious side effects on many important tissues. The kidney is very vulnerable to the side effects of TPL as an important excretory organ, which greatly limits the clinical application of TPL [47]. Therefore, the research on the toxicity reduction and efficiency enhancement of TPL is of great practical significance and needs to be further explored. It is the future research trend to transfer the mechanism of TPL on improving MF to clinical application.

Declarations

Competing interests

The authors declared that they have no competing interests.

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Author Contributions

JYS is the guarantor of integrity of the entire study; JYS contributed to the study concepts, study design, definition of intellectual content, literature research, manuscript preparation and manuscript editing and review; HLM contributed to the clinical studies; JYS and CQW contributed to the experimental studies and data acquisition; CQW contributed to the data analysis and statistical analysis. All authors read and approved the final manuscript.

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Availability of data and materials

All the data generated or analyzed during this study are included in this published article.

Patient consent for publication

Not applicable.

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Figures

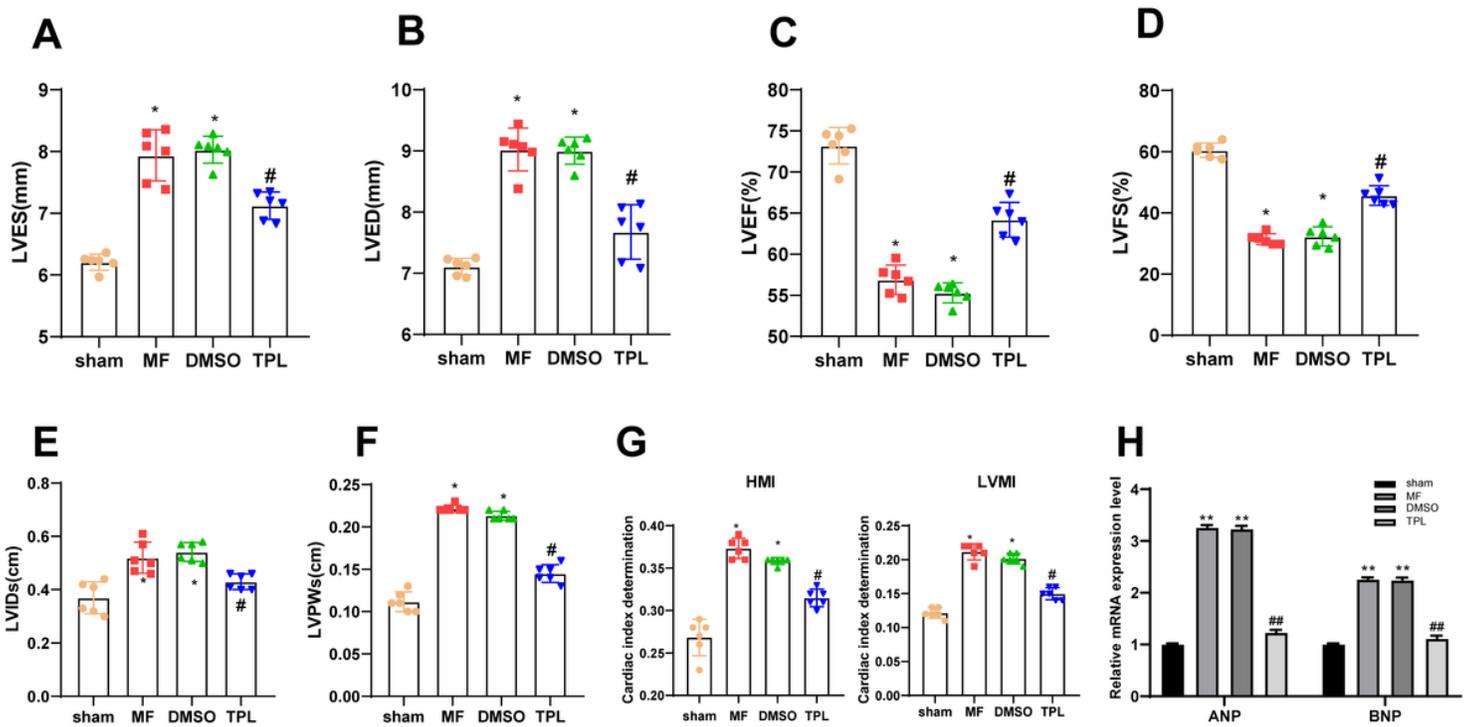


Figure 1

TPL improved cardiac dysfunction in MF rats. Myocardial fibrosis model was established by subcutaneous injection of ISO, and TPL was injected subcutaneously for 7 d. (A-D) The LVEF, LVFS, LVES and LVED were measured by the small animal ultrasonograph; (E) RT-qPCR was used to detect the ANP and BNP levels in myocardial tissues of rats in each group; N = 6. Three independent repeated tests were performed and the data were expressed as mean \pm standard deviation; one-way or two-way ANOVA was used for variance analysis; Tukey's multiple comparisons test was used for post hoc test. Compared with sham group, * $p < 0.05$, ** $p < 0.01$; compared with MF group, # $p < 0.05$, ## $p < 0.01$.

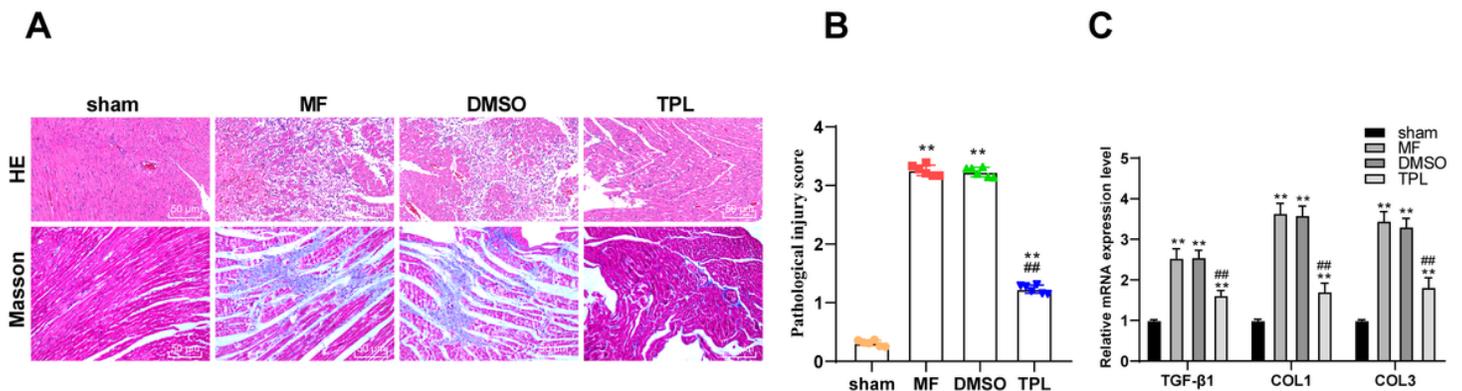


Figure 2

TPL downregulated the degree of MF and the expression of fibrosis related factors in MF rats. (A) HE staining and Masson staining were used to observe the pathological morphology and MF of rats in each

group; (B) Masson staining results were quantitatively analyzed by measuring collagen volume fraction; (C) RT-qPCR was used to detect the expressions of TGF- β 1, COL1 and COL3; (D) The expressions of TGF- β 1, COL1 and COL3 were detected by immunohistochemistry; N = 6. Three independent repeated tests were performed and the data were expressed as mean \pm standard deviation; one-way or two-way ANOVA was used for variance analysis; Tukey's multiple comparisons test was used for post hoc test. Compared with sham group, * p < 0.05, ** p < 0.01; compared with MF group, # p < 0.05, ## p < 0.01.

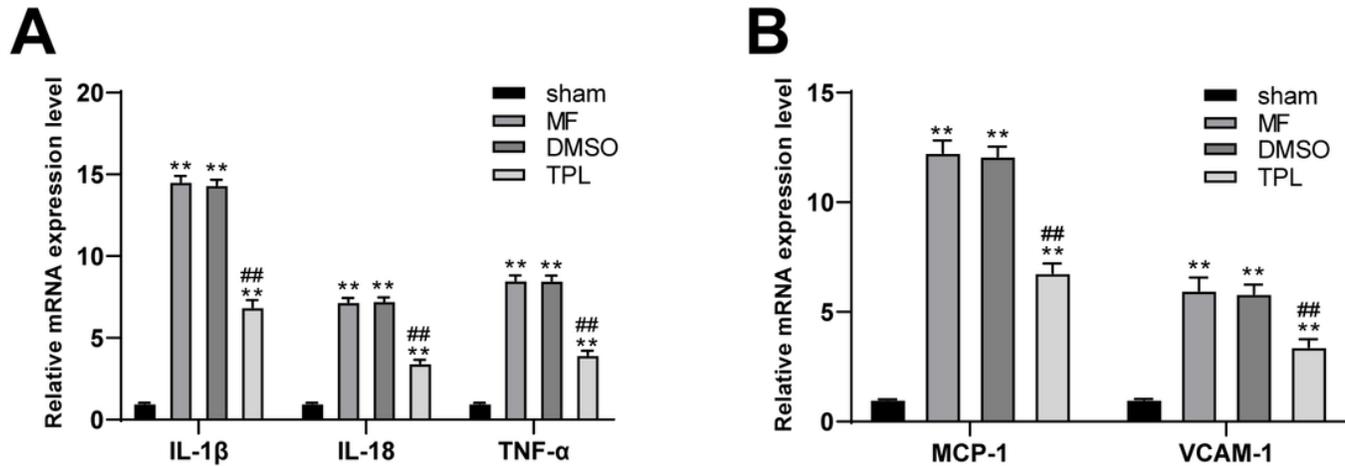


Figure 3

TPL downregulated the level of inflammatory factors in MF rats. (A-B) RT-qPCR and WB were used to respectively detect the mRNA and protein levels of inflammatory factors IL-1 β , IL-18 and TNF- α in the myocardium of rats; (C) RT-qPCR was used to detect the mRNA expression of MCP-1 and VCAM-1 in myocardium of rats in each group; N = 6. Three independent repeated tests were performed and the data were expressed as mean \pm standard deviation; two-way ANOVA was used for variance analysis; Tukey's multiple comparisons test was used for post hoc test. Compared with sham group, ** p < 0.01; compared with MF group, ## p < 0.01.

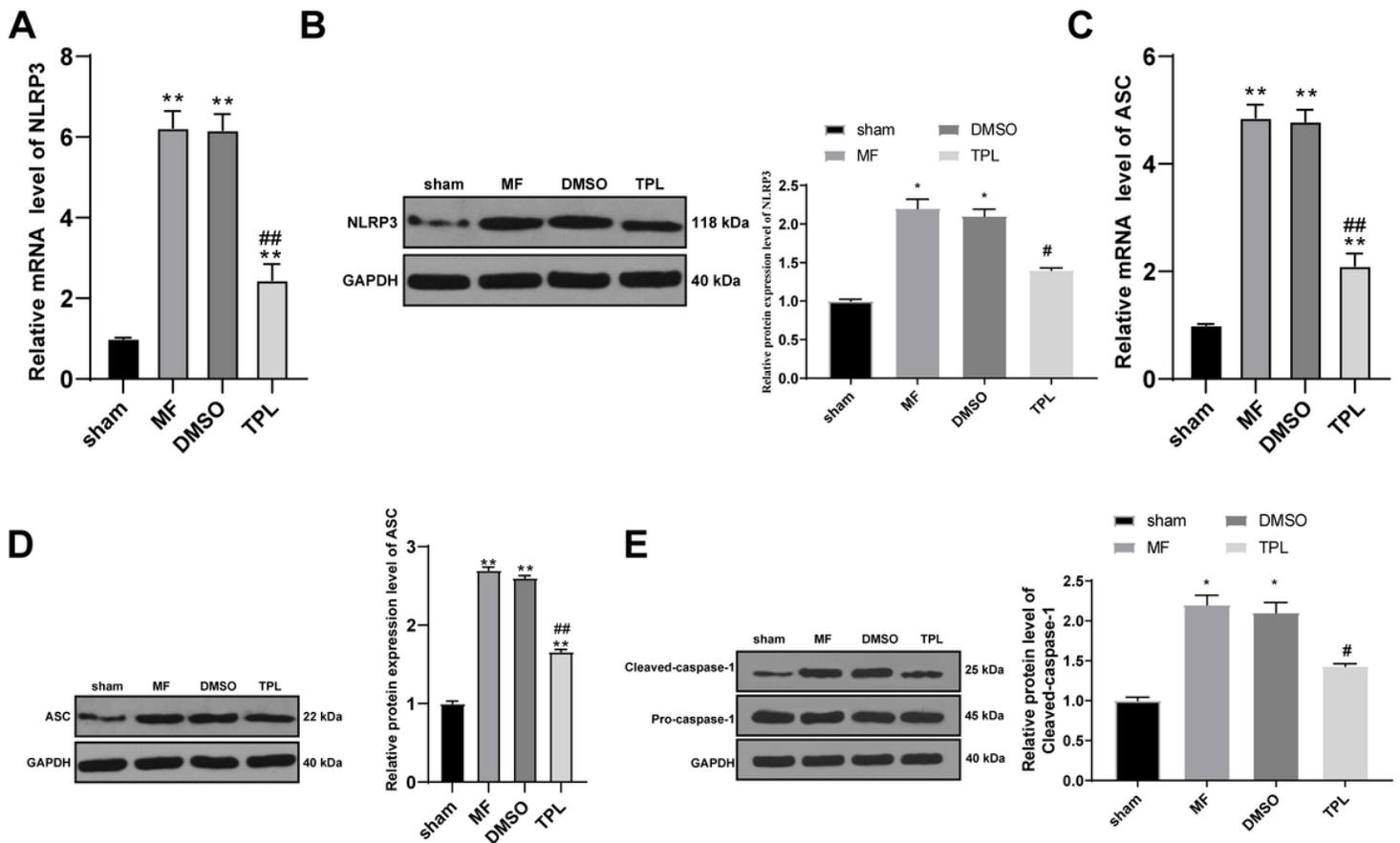


Figure 4

TPL inhibited the activation of NLRP3 inflammasome in MF rats. (A-D) RT-PCR and WB were used to detect the mRNA and protein levels of NLRP3 and ASC in MF rats; (E) The expressions of NLRP3 and ASC were detected by immunohistochemistry; N = 6. Three independent repeated tests were performed and the data were expressed as mean \pm standard deviation; one-way ANOVA was used for variance analysis; Tukey's multiple comparisons test was used for post hoc test. Compared with sham group, ** $p < 0.01$; compared with MF group, ## $p < 0.01$.

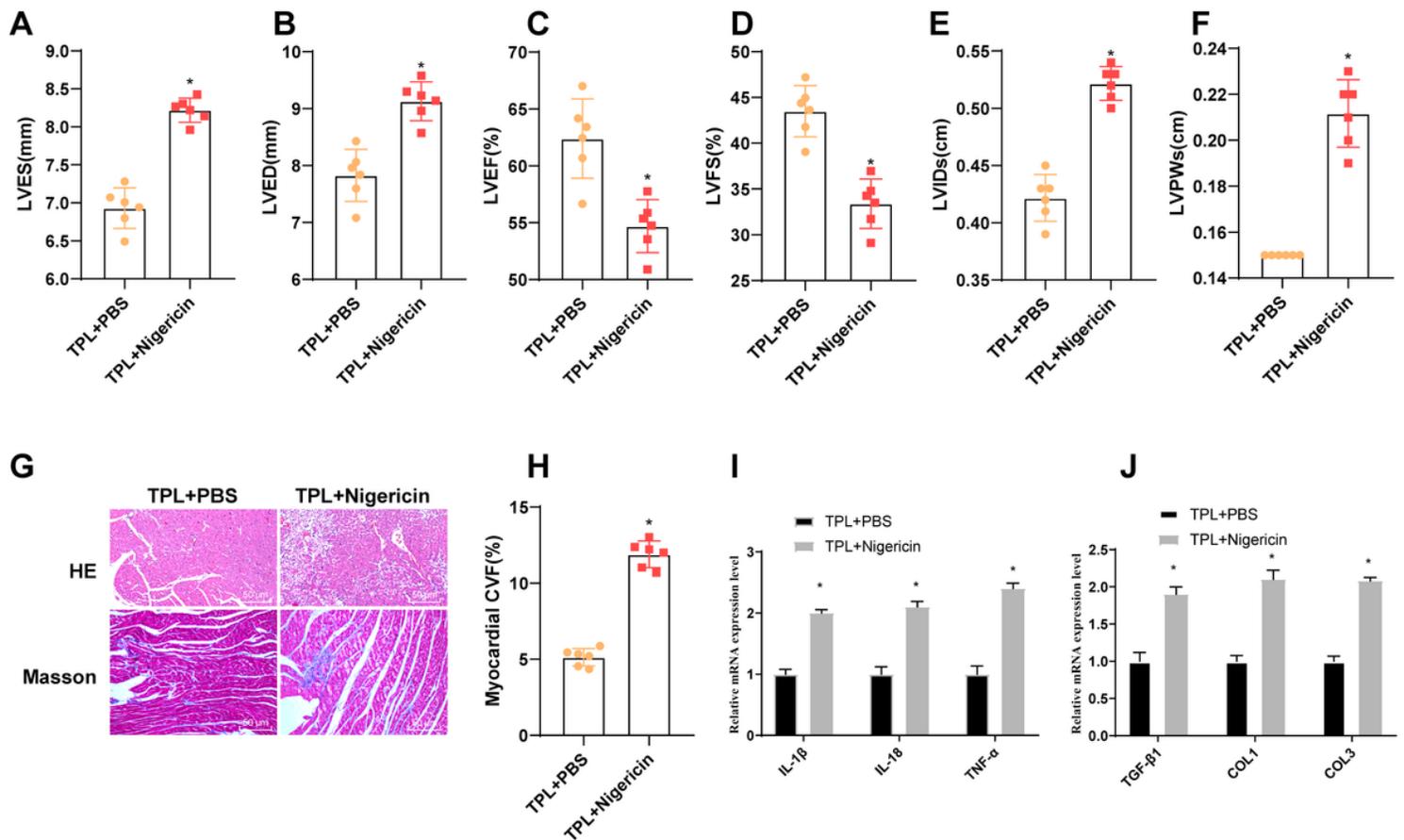


Figure 5

TPL improved the degree of MF in MF rats by inhibiting the activation of NLRP3 inflammasome pathway. (A) RT-qPCR was used to detect the mRNA expressions of inflammatory factors IL-1 β , IL-18 and TNF- α in myocardial tissues; (B) RT-qPCR was used to detect the mRNA expressions of TGF- β 1, COL1 and COL3; (C) The expressions of TGF- β 1, COL1 and COL3 were detected by immunohistochemistry; N = 6. Three independent repeated tests were performed and the data were expressed as mean \pm standard deviation; two-way ANOVA was used for variance analysis; Tukey's multiple comparisons test was used for post hoc test. Compared with TPL + PBS group, **p < 0.01.

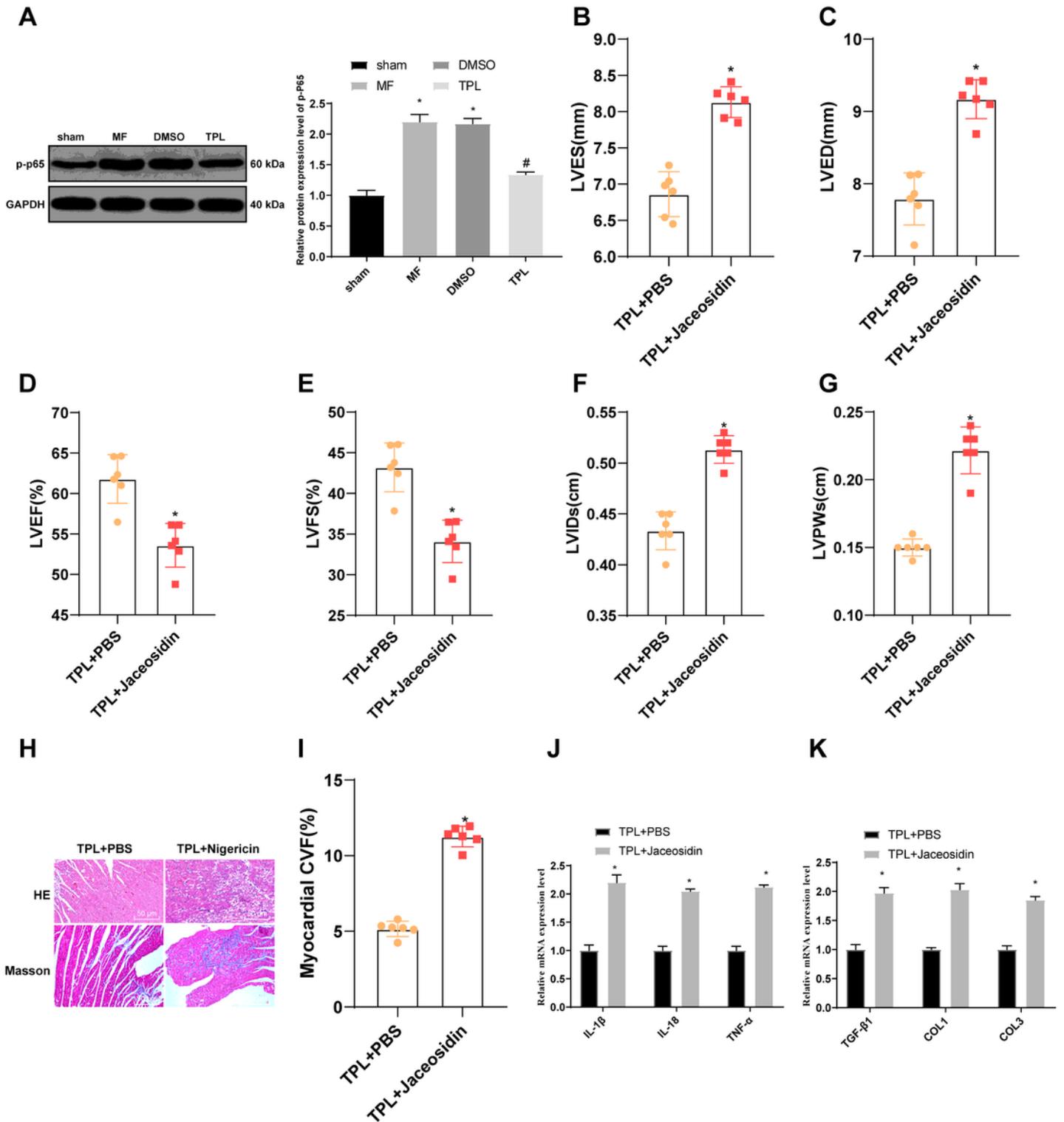


Figure 6

TPL inhibited the activation of NLRP3 inflammasome by inhibiting NF- κ B pathway and improved the degree of MF. (A) WB was used to detect the phosphorylation of P65 in myocardium of rats in each group; (B) RT-qPCR was used to detect the mRNA expressions of inflammatory factors IL-1 β , IL-18 and TNF- α in myocardial tissues of rats in each group; (C) RT-qPCR was used to detect the mRNA expressions of fibrosis related factors TGF- β 1, COL1 and COL3 in myocardium; N = 6. Three independent repeated

tests were performed and the data were expressed as mean \pm standard deviation; one-way or two-way ANOVA was used for variance analysis; Tukey's multiple comparisons test was used for post hoc test. Compared with TPL + PBS group, **p < 0.01.