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# 5-Methoxytryptophan pretreatment alleviates lipopolysaccharide-induced cardiac injury and dysfunction

Yang Fu<sup>1,2</sup> and Yi-Fei Dong<sup>1,2\*</sup>

## Abstract

Reducing inflammation is a promising therapeutic approach for sepsis-induced cardiomyopathy (SIC). The 5-Methoxytryptophan (5-MTP) is a tryptophan metabolite that demonstrates anti-inflammatory, anti-fibrosis, anti-tumorigenesis, and anti-senescence features. Current investigations aimed to assess the 5-MTP pretreatment impacts on lipopolysaccharide (LPS)-induced cardiac injury and dysfunction. For in vivo studies, the mice were categorized randomly into four groups: control, LPS, LPS+5-MTP (25 mg/kg) and LPS+5-MTP (50 mg/kg). The mice in the LPS+5-MTP groups were given 5-MTP intraperitoneally once a day for 7 days. LPS (10 mg/kg) was then administered intraperitoneally for 24 h. Echocardiography, cardiac injury biomarkers, and H & E staining evaluated heart anatomy and function. The findings indicate that 5-MTP pretreatment significantly reduced LPS-induced heart dysfunction and morphological alterations. Western blot assay was used for investigating molecular mechanisms. After LPS stimulation, the pro-inflammatory cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$  and NLRP3) protein levels increased while anti-inflammatory cytokine (IL-10) decreased; however, 5-MTP pretreatment mitigated this response by suppressing the stimulation of the NF- $\kappa$ B signaling pathway. Furthermore, 5-MTP administration reduced LPS-induced cardiac apoptosis, as demonstrated by increased protein levels of cleaved-Caspase-1, cleaved-Caspase-3 and Bax, and decreased protein level of Bcl-2 after LPS stimulation, whereas LPS-induced cardiac apoptosis was reversed by 5-MTP pretreatment. In vitro, 5-MTP pretreatment had a similar cardioprotective effect on cultured cardiac fibroblasts challenged with LPS. In conclusion, 5-MTP pretreatment can reduce LPS-induced cardiac inflammation and apoptosis, implying that 5-MTP is a possible therapeutic option for SIC.

**Keywords:** Sepsis-induced cardiomyopathy (SIC), 5-Methoxytryptophan (5-MTP), Lipopolysaccharide (LPS), Inflammation, Apoptosis

## Introduction

The entry of different bacteria or other harmful microbes into the body causes sepsis, a systemic inflammatory condition, contributing to high morbidity and mortality worldwide [1]. In patients with severe sepsis, SIC (sepsis-induced cardiomyopathy) appears easily and is defined as cardiac injury and dysfunction [2]. The pathological

characteristics of SIC include inflammation, apoptosis, fibrosis, and oxidative stress in heart tissue [3, 4]. Thus, reducing inflammation might be a promising therapeutic approach for SIC. Despite symptomatic and supportive treatments, there are still no standard therapeutic approaches for SIC at present.

5-Methoxytryptophan (5-MTP), originally discovered as a cyclooxygenase-2 (COX-2) inhibitor in the conditioned medium of fibroblasts and then named cytoguardin, acts as a tryptophan metabolite and can be found both in humans and animal [5, 6]. The synthesis of this compound includes two steps: first, the tryptophan is

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hydroxylated by tryptophan hydroxylase to make up of 5-hydroxytryptophan; next, the 5-hydroxytryptophan is methylated by hydroxyindole-O-methyltransferase (HIOMT) and eventually forms 5-MTP [6, 7]. A variety of cells was found to synthesize 5-MTP, like fibroblasts, cancer cells, bronchial epithelial cells, renal epithelial cells, smooth muscle cells, vascular endothelial cells, and bronchial epithelial cells [8]. Previous studies have found that 5-MTP has anti-tumorigenesis, anti-fibrosis, vascular protection, anti-inflammation, and anti-senescence characteristics in various disease models [9–12]. However, the specific effect of 5-MTP on LPS-induced cardiac injury and dysfunction is still unknown.

The current study hypothesizes that 5-MTP can alleviate LPS-induced cardiac injury and dysfunction. To verify this hypothesis, we investigated the effect of 5-MTP on the mice with the SIC model. Besides, we further evaluated the effect of 5-MTP on cultured cardiac fibroblasts (CFs) challenged with LPS. In the present study, we detected the cardiac function impairment relevant indicators (like echocardiography, serum CK-MB and LDH levels, and H & E staining), the inflammatory cytokines (like IL-1 $\beta$ , IL-6, IL-10, TNF- $\alpha$ , and NLRP3), as well as the apoptosis-related proteins (like cleaved-Caspase-1, cleaved-Caspase-3, Bax, and Bcl-2).

## Materials and methods

### Materials

Hunan Slac Jingda Laboratory Animal Co., Ltd. (Changsha, China) provided 32 (age=6 weeks) male C57BL/6 mice, weighing  $22 \pm 3$  g. The Chinese Academy of Medical Science (Shanghai, China) provided cardiac fibroblasts (CFs) for this study. Two crucial reagents applied to this study were as follows: DL-5-methoxytryptophan (5-MTP, Sigma Co., Ltd., St. Louis, MO, USA) and lipopolysaccharide (LPS, Sigma Co., Ltd., St. Louis, MO, USA). The remaining reagents included in this investigation were standard and easily available.

### Animal treatment

All mice in this study were maintained in a sterile environment (20 °C and 60% humidity in 12 h light–dark cycle) with accessibility to food and water at Nanchang University's Jiangxi Medical Animal Care Center. Mice were fed for around 2 weeks before any experimental treatments to help them adjust to their new surroundings. Then they were categorized randomly (eight in each group) into control, LPS, LPS+5-MTP (25 mg/kg) and LPS+5-MTP (50 mg/kg) groups. (1): the mice in the control group were given phosphate-buffered saline (PBS) and no other chemicals. (2): the mice in the LPS group were injected with PBS intraperitoneally (i.p.) once per day for 7 days, and then followed by 10 mg/kg

of LPS intraperitoneally (i.p.) once for another 24 h. (3): the mice in the LPS+5-MTP(25 mg/kg) group were given 5-MTP (25 mg/kg) intraperitoneally (i.p.) once every day for over 7 days, following LPS (10 mg/kg, i.p.) for another 24 h. (4): the mice in the LPS+5-MTP(50 mg/kg) group were given 5-MTP (50 mg/kg) intraperitoneally (i.p.) once every day for over 7 days, following LPS (10 mg/kg, i.p.) for another 24 h. The methods and the procedures of reagents administration applied to this study were in line with previous studies [13]. The Animal Care and Use Committee of Nanchang University's Second Affiliated Hospital authorized the current experimentations, and all experimental procedures in mice were carried out in compliance with legal requirements.

### Echocardiography

The cardiac function of the mice in this study was evaluated by echocardiography and the procedures of echocardiography were as follows. 1.5% of isoflurane was applied to anesthetize the mice before being tethered to a heating pad in a reclined position. We used a Vevo770 device (Visual-Sonics, Canada) with a 30-Hz transducer for transthoracic echocardiography after the mice's body hair was removed. Left ventricle end-systolic diameter (LVESD) and left ventricular end-diastolic diameter (LVEDD) were measured and used to calculate left ventricle ejection fraction (LVEF %) and left ventricle shortening fraction (LVSF %). Following the completion of these performances, the mice were instantly slaughtered, and for subsequent study, the heart tissues were removed.

### Measurement of serum CK-MB and LDH levels

We employed pentobarbital sodium (30 mg/kg, i.p.) to anesthetize these mice after echocardiography monitoring was done, and then these mice were slaughtered. A vacuum tube was applied to collect whole blood from the right ventricle of the mice. Subsequently, the whole blood collected from the mice was used to extract the serum at a centrifugation of 4000 rpm for around 30 min, with an operating temperature of 4 °C. An automatic biochemical analyzer (Chemray240, Rayto, China) was used to detect serum levels of heart damage biomarkers CK-MB and LDH.

### Measurement of serum inflammatory cytokines by ELISA (enzyme-linked immunosorbent assay)

Specific mice ELISA kits were utilized to measure inflammatory cytokines such as IL-1 $\beta$ , IL-6 and TNF- $\alpha$  levels in the blood. The ELISA kits' details were as follows: TNF- $\alpha$  (Catalogue No. 88-7324, Invitrogen, California, USA), IL-6 (Catalogue No. 88-7064, Invitrogen, California, USA), and IL-1 $\beta$  (Catalogue No. 88-7013, Invitrogen,

California, USA). All experiments were conducted under manufacturers provided guidelines.

### H&E (hematoxylin and Eosin) staining

Heart tissues were isolated from the mice immediately when they were sacrificed. The heart tissue was then sliced into three sections, and the central section was preserved in a 4% formaldehyde solution for roughly an hour. Following that, this piece of heart tissue was cut into 5  $\mu$ m portions at several depths for an H & E staining assay. The detailed procedures in this assay were in line with standard experimental operational instructions.

### Culture and treatment of mice CFs

At a density of  $1 \times 10^6$  cells, the mice CFs were seeded in a 6 cm diameter cell culture dish that was filled with 3 ml cells culture medium. The cells culture medium was composed of DMEM (Dulbecco's modified Eagle's medium, Thermo Fisher, USA), 10% FBS (fetal bovine serum, Gibco, USA) and 1% streptomycin and penicillin (Hyclone). All of these cells were cultured at 37 °C with 5% CO<sub>2</sub> in a humidified incubator. The mice CFs were pretreated for approximately 12 h with 5-MTP at low, medium, and high (5  $\mu$ M, 10  $\mu$ M, 50  $\mu$ M, respectively) concentrations and subsequently activated with 100 ng/ml LPS for a later 24 h. These cells were randomly allocated into five groups in the current study: (1) Control group: without any other reagents treatments; (2) LPS group: stimulation of LPS (100 ng/ml); (3) LPS+5-MTP (5  $\mu$ M) group: pretreatment of 5-MTP (5  $\mu$ M) and stimulation of LPS (100 ng/ml); (4) LPS+5-MTP (10  $\mu$ M) group: pretreatment of 5-MTP (10  $\mu$ M) and stimulation of LPS (100 ng/ml); (5) LPS+5-MTP (50  $\mu$ M) group: pretreatment of 5-MTP (50  $\mu$ M) and stimulation of LPS (100 ng/ml). Following these treatments, the mice CFs in every group were collected for subsequent investigation of the molecular processes.

### Western blot assay

Total proteins from mice cardiac tissues were isolated in the current study through a protein extraction kit purchased from Beyotime Biotechnology (Catalogue No. P0013B, Jiangsu, China). Before any performances of western blot assay, we applied a bicinchoninic-acid (BCA) protein estimation kit (Catalogue No. P0012, Beyotime Biotechnology, Jiangsu, China) to detect the protein quantities. To separate the total proteins from each sample, 10% sodium-dodecyl sulfate–polyacrylamide gel-electrophoresis (SDS-PAGE) was utilized, and the proteins were then deposited into PVDF-membranes (EMD-Millipore, MA, USA). Following that, these PVDF-membranes were treated with specific primary antibodies and incubated for approximately 12 h at 4 °C, followed by exposure

to appropriate secondary antibodies for around 1 h at room temperature. In addition, we used an advanced chemiluminescence detection kit and a scanner (ThermoFisher-Scientific, MA, USA) to measure protein bands in PVDF membranes. In the present study, the detailed information of primary antibodies were as follows: anti-IL-1 $\beta$  (Catalogue No. ab-200478; 1:1000; Abcam, Cambridge, MA, USA), anti-IL-6 (Catalogue No. ab-6672; 1:1000; Abcam, Cambridge, MA, USA), anti-TNF- $\alpha$  (Catalogue No. ab-1793; 1:1000; Abcam, Cambridge, MA, USA), anti-IL-10 (Catalogue No. ab-52909; 1:1000; Abcam, Cambridge, MA, USA), anti-NLRP3 (Catalogue No. 19771-1-AP; 1:1000; Proteintech Rosemont, IL, USA), anti-phosphorylated (p)-NF- $\kappa$ B (Catalogue No. CST-3033S; 1:1000; Cell-Signaling-Technology, Danvers, MA, USA), anti-NF- $\kappa$ B (Catalogue No. CST-8242S; 1:1000; Cell-Signaling-Technology, Danvers, MA, USA), anti-cleaved-Caspase-1 (Catalogue No. CST-89332S; 1:1000; Cell-Signaling-Technology, Danvers, MA, USA), anti-Caspase-1 (Catalogue No. ab-138483; 1:1000; Abcam, Cambridge, MA, USA), anti-cleaved-Caspase-3 (Catalogue No. ab-32042; 1:1000; Abcam, Cambridge, MA, USA), anti-Caspase-3 (Catalogue No. ab-44976; 1:1000; Abcam, Cambridge, MA, USA), anti-Bax (Catalogue No. ab-32503; 1:1000; Abcam, Cambridge, MA, USA), anti-Bcl-2 (Catalogue No. ab-182858; 1:1000; Abcam, Cambridge, MA, USA), anti-GAPDH (Catalogue No. 60004-1-Ig; 1:1000; Proteintech Rosemont, IL, USA). Secondary antibodies includes goat anti-mouse-IgG (Catalogue No. 15014; 1:5000; Proteintech Rosemont, IL, USA) and goat anti-rabbit-IgG (Catalogue No. B900210; 1:5000; Proteintech Rosemont, IL, USA). At least three times in separate experiments, the western blot assay was conducted. All findings were examined using Image-Lab (version: 4.0.1) software. In the present study, the relative target protein expression levels in each group were obtained by dividing the target protein levels by the GAPDH protein level.

### Statistical analyses

The results of this investigation are provided as means and standard deviations (means  $\pm$  SD). For all statistical analyses, the GraphPad Prism 7.0 software (GraphPad Software Inc., San Diego, CA, USA) was utilized. To compare different groups in the current study, ANOVA (analysis of variance) was used, with a P-value of less than 0.05 considered as statistically significant, as \* denote *p*-values < 0.05.

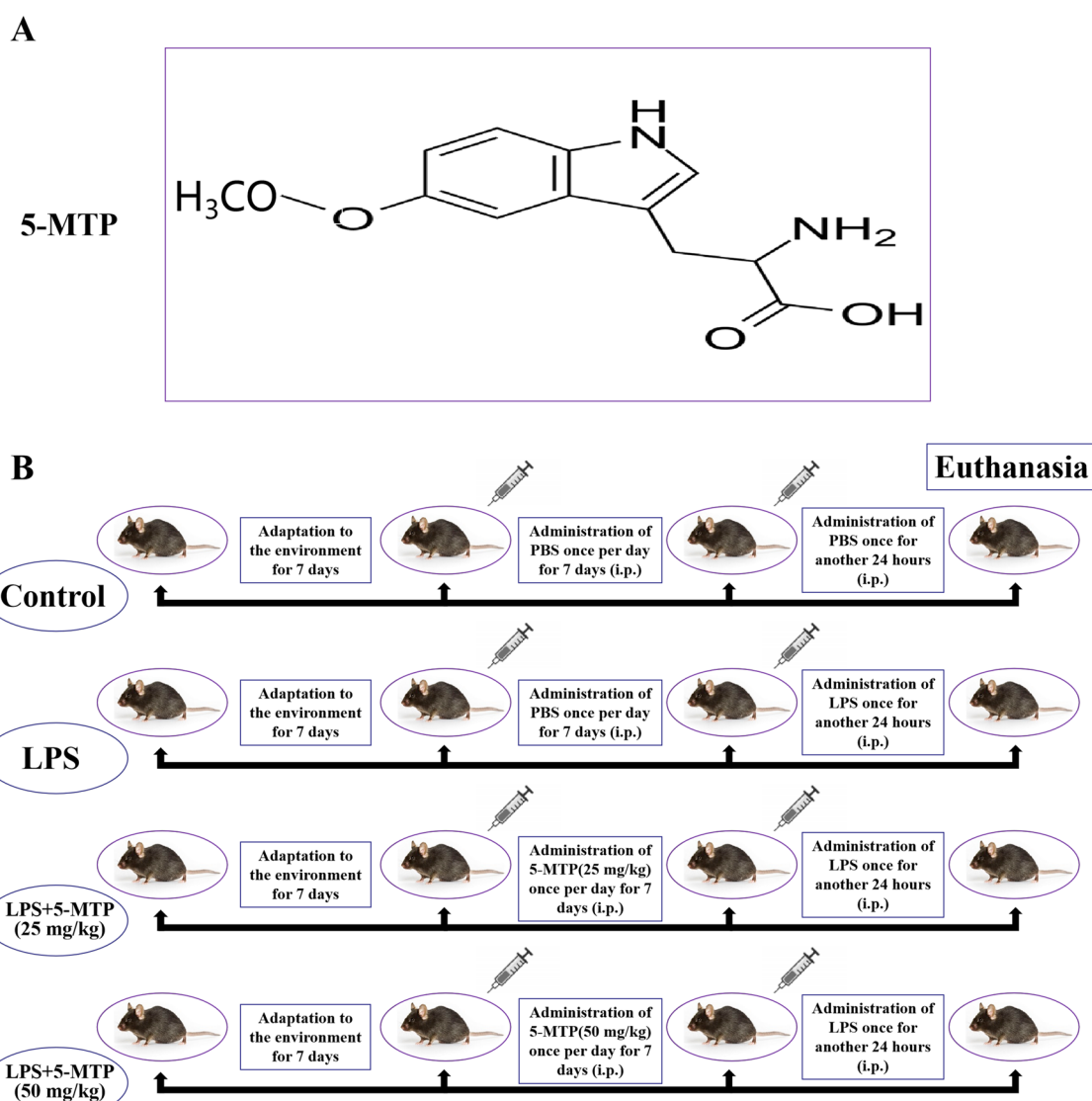
## Results

### 5-MTP pretreatment reduced LPS-induced cardiac injury and dysfunction in mice

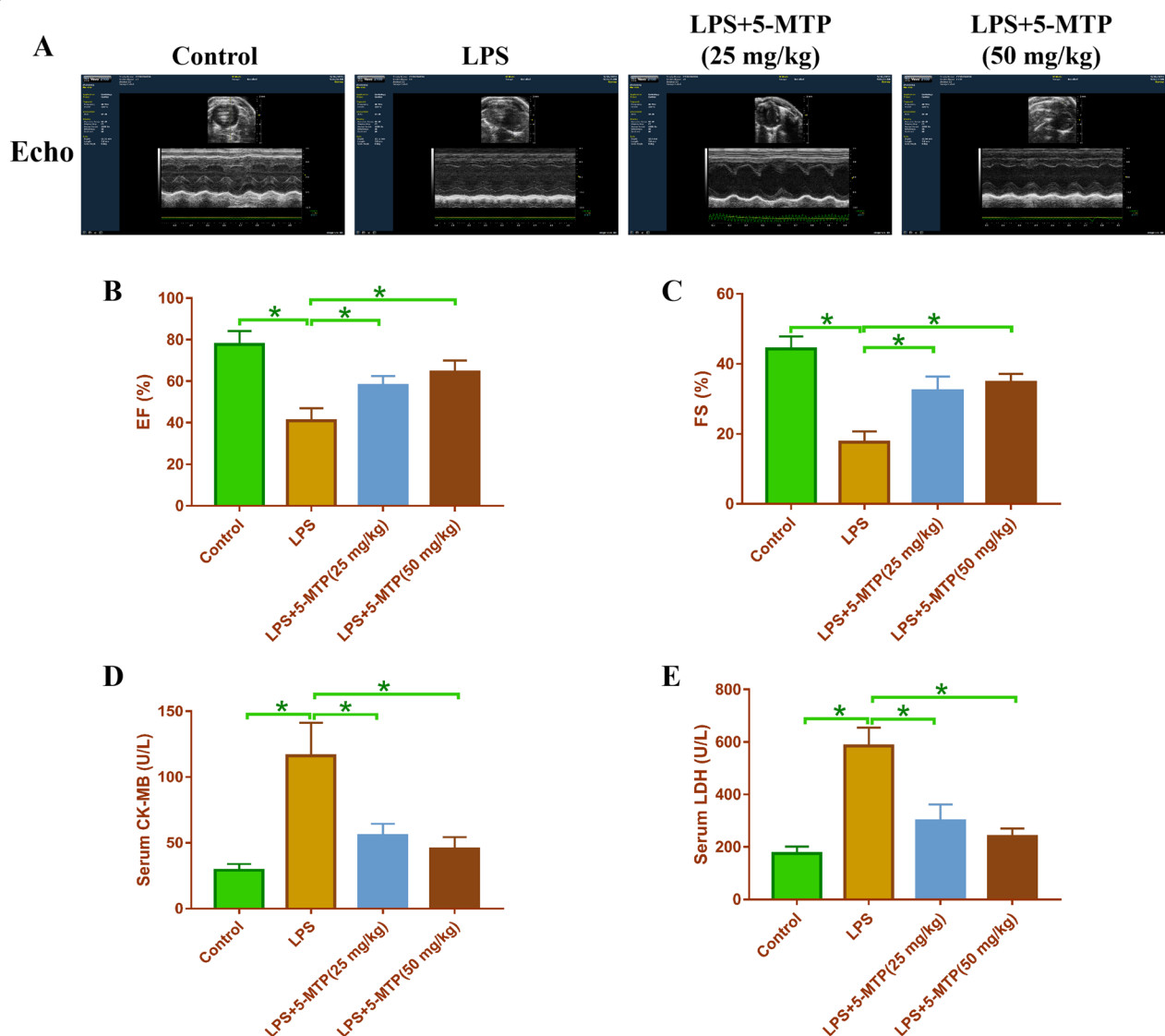
The chemical structure of 5-MTP and animal treatments procedures were summarized in Fig. 1A and B. Echocardiography, cardiac damage biomarkers (LDH and

CK-MB), and pathological changes were used to assess the heart function of mice. First, indicators of echocardiography, like left ventricle ejection fraction (LVEF, EF, %) and left ventricle fractional shortening (LVFS, FS, %), significantly decreased ( $p < 0.05$ ) after LPS stimulation. When compared to the LPS group, 5-MTP pretreatment improved ( $p < 0.05$ ) these indicators in the LPS+5-MTP (25 mg/kg) and LPS+5-MTP (50 mg/kg) groups (Fig. 2A–C). Additionally, we measured the cardiac injury biomarkers (LDH and CK-MB) levels in the serum and discovered that LPS stimulation significantly increased ( $p < 0.05$ ) the LDH and CK-MB serum levels, whereas 5-MTP pretreatment markedly reversed

( $p < 0.05$ ) these effects in the LPS+5-MTP (25 mg/kg) and LPS+5-MTP (50 mg/kg) groups in comparison to the LPS group (Fig. 2D and E). H&E staining was conducted to evaluate pathological changes in heart tissues. The findings indicate that, in comparison to the control group, the LPS group experienced an increase in heart pathological damage. However, when 5-MTP was added to the mice, this change was considerably improved in the LPS+5-MTP (25 mg/kg) and LPS+5-MTP (50 mg/kg) groups in comparison to the LPS group (Fig. 3A). All of these findings suggested that pretreatment with 5-MTP ameliorated LPS-induced cardiac injury and dysfunction in mice.



**Fig. 1** **A**, the chemical structure of 5-MTP. **B**, the treatment procedures in different groups of mice



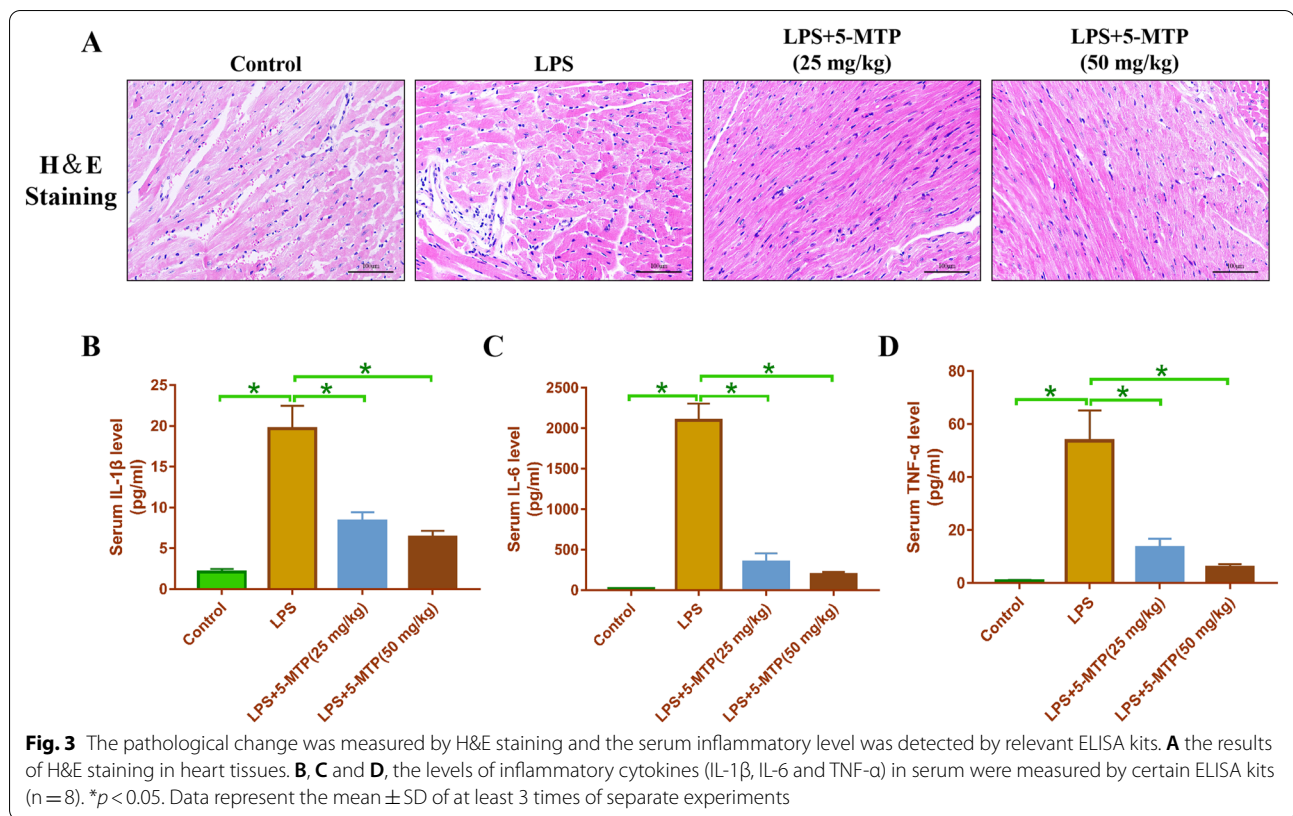
**Fig. 2** The cardiac function of mice was evaluated by echocardiography and cardiac injury biomarkers. **A**, a image of echocardiography in mouse left ventricle. **B, C**, echocardiographic analysis of left ventricle ejection fraction (LVEF, EF, %) and left ventricle fractional shortening (LVFS, FS, %) (n = 8). **D, E**, the serum levels of cardiac injury biomarkers (CK-MB and LDH) (n = 8). \* $p < 0.05$ . Data represent the mean  $\pm$  SD of at least 3 times of separate experiments

### Pretreatment with 5-MTP inhibited LPS-induced myocardial inflammation in mice

An inappropriate inflammatory response is a significant contributor to the pathological alterations in the heart caused by LPS. To determine the effect of 5-MTP pretreatment on the aberrant inflammatory response generated by LPS, we used an enzyme-linked immunosorbent assay and a western blot assay to evaluate the amounts of pro-inflammatory cytokines (NLRP3, IL-6, IL-1 $\beta$ , and TNF- $\alpha$ ) and anti-inflammatory cytokine (IL-10) in blood and heart tissue. Compared with the control group, the

levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  in serums significantly increased ( $p < 0.05$ ) after LPS stimulation in the LPS group, while 5-MTP pretreatment inhibited ( $p < 0.05$ ) these effects in the LPS+5-MTP(25 mg/kg) and LPS+5-MTP(50 mg/kg) groups (Fig. 3B–D). Similarly, when comparing the LPS group to the control group, the protein expression levels of IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and NLRP3 in heart tissues were increased ( $p < 0.05$ ), but pretreatment with 5-MTP substantially reduced ( $p < 0.05$ ) corresponding indices in the LPS+5-MTP (25 mg/kg) and LPS+5-MTP (50 mg/kg) groups (Fig. 4A–D). IL-10, an





anti-inflammatory cytokine, was found to decrease after LPS stimulation alone, whereas it was increased when combined with 5-MTP pretreatment (Fig. 4E). As previously stated, the NF- $\kappa$ B signaling pathway is capable of regulating inflammatory responses. As a result, we study the effect of 5-MTP administration on the NF- $\kappa$ B signaling pathway stimulation induced by LPS. The results showed that pretreatment with 5-MTP reduced the LPS-stimulated phosphorylation of NF- $\kappa$ B (Fig. 4F). These findings suggested that pretreatment with 5-MTP could limit LPS-induced heart inflammation in mice by reducing the NF- $\kappa$ B signaling pathway activation.

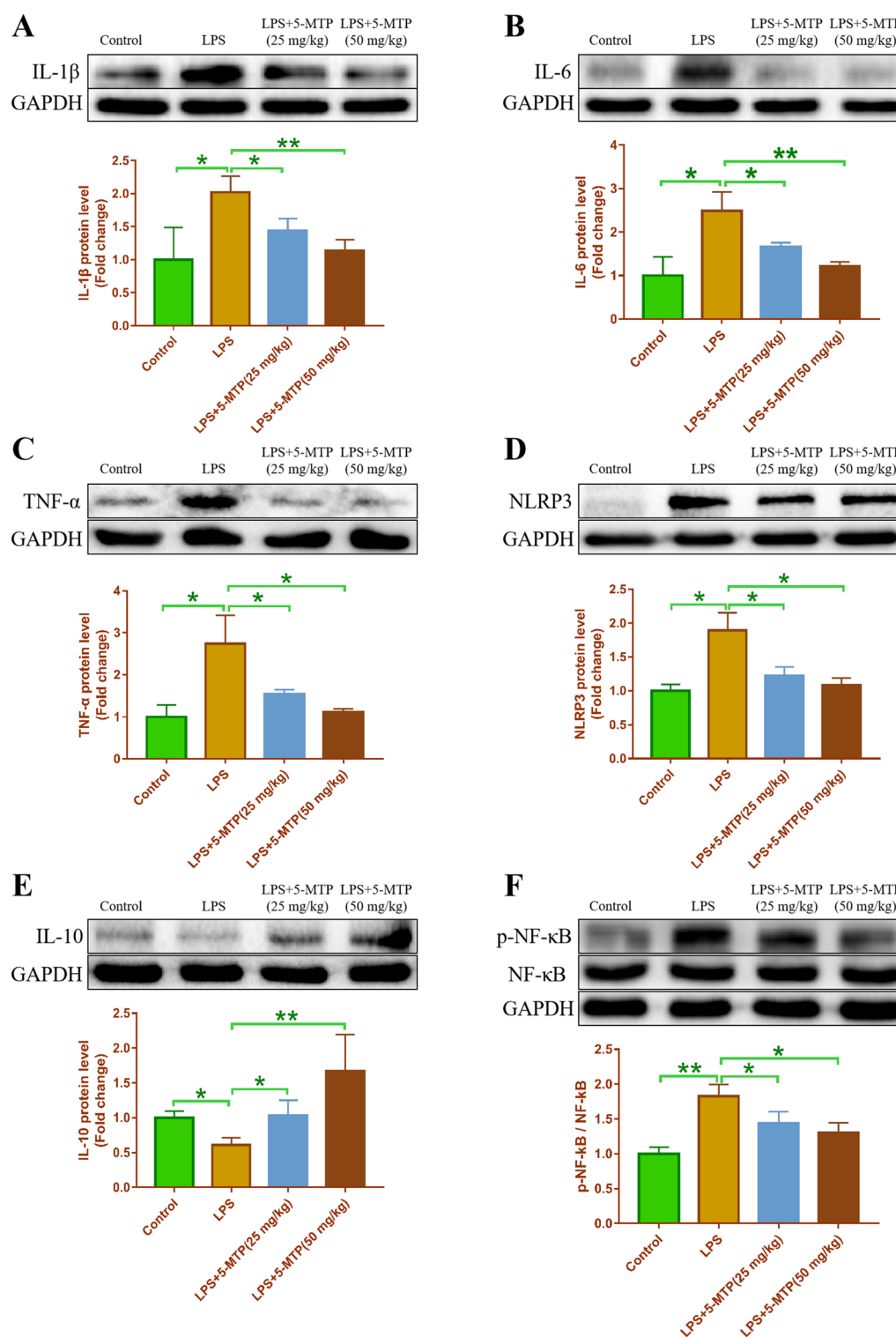
#### 5-MTP pretreatment inhibited LPS-induced cardiac apoptosis in mice

To determine the impact of 5-MTP pretreatment on LPS-induced cardiac apoptosis, we measured the levels of apoptosis-related proteins such as cleaved-Caspase-1, cleaved-Caspase-3, Bax, and Bcl-2. cleaved-Caspase-1, cleaved-Caspase-3, and Bax protein levels were considerably enhanced ( $p$  < 0.05) in the LPS group compared to the control mice, but these effects were suppressed ( $p$  < 0.05) by 5-MTP pretreatment in the LPS+5-MTP (25 mg/kg) and LPS+5-MTP (50 mg/kg) groups (Fig. 5A–C). On the other hand, the Bcl-2 protein level was considerably lowered ( $p$  < 0.05) in the LPS group,

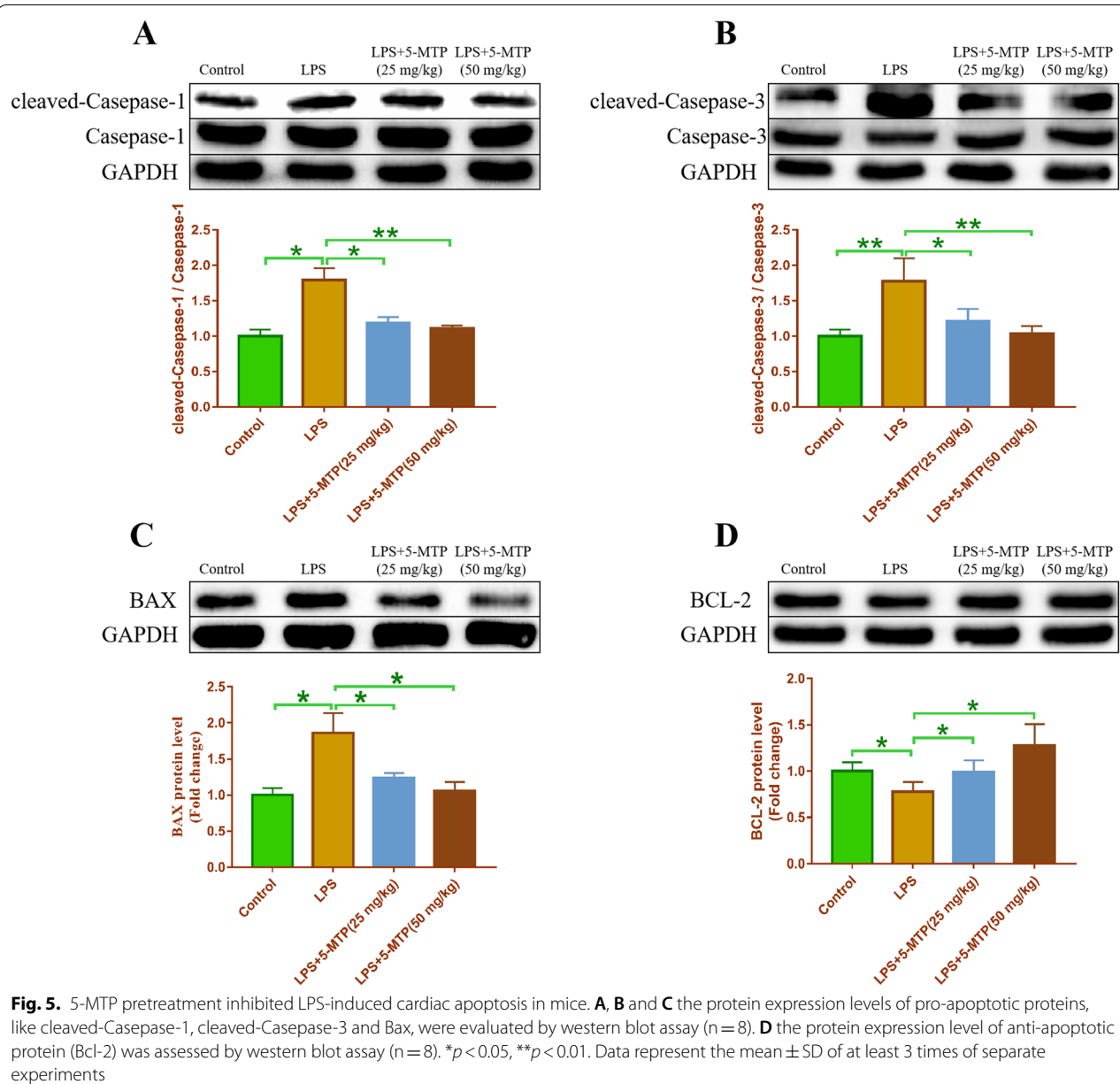
but this effect was enhanced ( $p$  < 0.05) in the LPS+5-MTP (25 mg/kg) and LPS+5-MTP (50 mg/kg) groups (Fig. 5D). These findings demonstrated that 5-MTP pretreatment inhibited LPS-induced myocardial apoptosis in mice.

#### 5-MTP pretreatment attenuated LPS-induced inflammation in cultured CFs

The mice CFs were pretreated with low, medium, and high concentrations (5  $\mu$ M, 10  $\mu$ M, and 50  $\mu$ M respectively) of 5-MTP for 12 h before 100 ng/ml administration of LPS for additional 24 h. The effect of 5-MTP pretreatment on LPS-induced inflammatory response in cultured CFs were investigated. When comparing the LPS-treated CFs to the control group, the proteins levels of pro-inflammatory cytokines (TNF- $\alpha$  and NLRP3) enhanced ( $p$  < 0.05) and anti-inflammatory cytokine (IL-10) decreased ( $p$  < 0.05), as demonstrated by Western blot analysis, however, these effects were reversed by 5-MTP pretreatment (Fig. 6A–C). Additionally, 5-MTP pretreatment was found to inhibit LPS-induced NF- $\kappa$ B signaling pathway activation (Fig. 6D). The findings showed that inhibiting the NF- $\kappa$ B signaling pathway with 5-MTP reduced the LPS-induced inflammation in cultured CFs.



**Fig. 4.** 5-MTP pretreatment alleviated LPS-induced cardiac inflammation by inhibiting the activation of NF-κB signaling pathway in mice. **A–E**, western blot analysis was applied to detecting the protein levels of inflammatory cytokines (IL-1β, IL-6 and TNF-α), NLRP3 inflammasome and anti-inflammatory cytokine (IL-10) in mouse heart tissues (n=8). **F**, the change of protein expression level of p-NF-κB was investigated by western blot assay (n=8). \* $p < 0.05$ , \*\* $p < 0.01$ . Data represent the mean  $\pm$  SD of at least 3 times of separate experiments



### LPS-induced apoptosis in cultured CFs was reduced by 5-MTP pretreatment

We evaluated the protein levels of apoptosis-related proteins, such as Bax and Bcl-2, in cultured CFs to further validate the influence of 5-MTP pretreatment on

LPS-induced apoptosis. Western blotting results indicated that when the LPS group was compared to the control group, the pro-apoptotic protein level of BAX increased ( $p < 0.05$ ), while 5-MTP pretreatment mitigated this effect (Fig. 6E). In the LPS group, however,

(See figure on next page.)

**Fig. 6.** 5-MTP pretreatment reduced LPS-induced inflammation and apoptosis in cultured CFs. **A, B** and **C** western blot assay was used for measuring the protein levels of inflammatory cytokine (TNF- $\alpha$ ), NLRP3 inflammasome and anti-inflammatory cytokine (IL-10) in cultured CFs ( $n = 8$ ). **D**, the change of protein expression level of p-NF- $\kappa$ B in cultured CFs was investigated by western blot assay ( $n = 8$ ). **E** and **F**, the protein levels of pro-apoptotic protein (Bax) and anti-apoptotic protein (Bcl-2) were detected by western blot assay ( $n = 8$ ). \* $p < 0.05$ , \*\* $p < 0.01$ . Data represent the mean  $\pm$  SD of at least 3 times of separate experiments



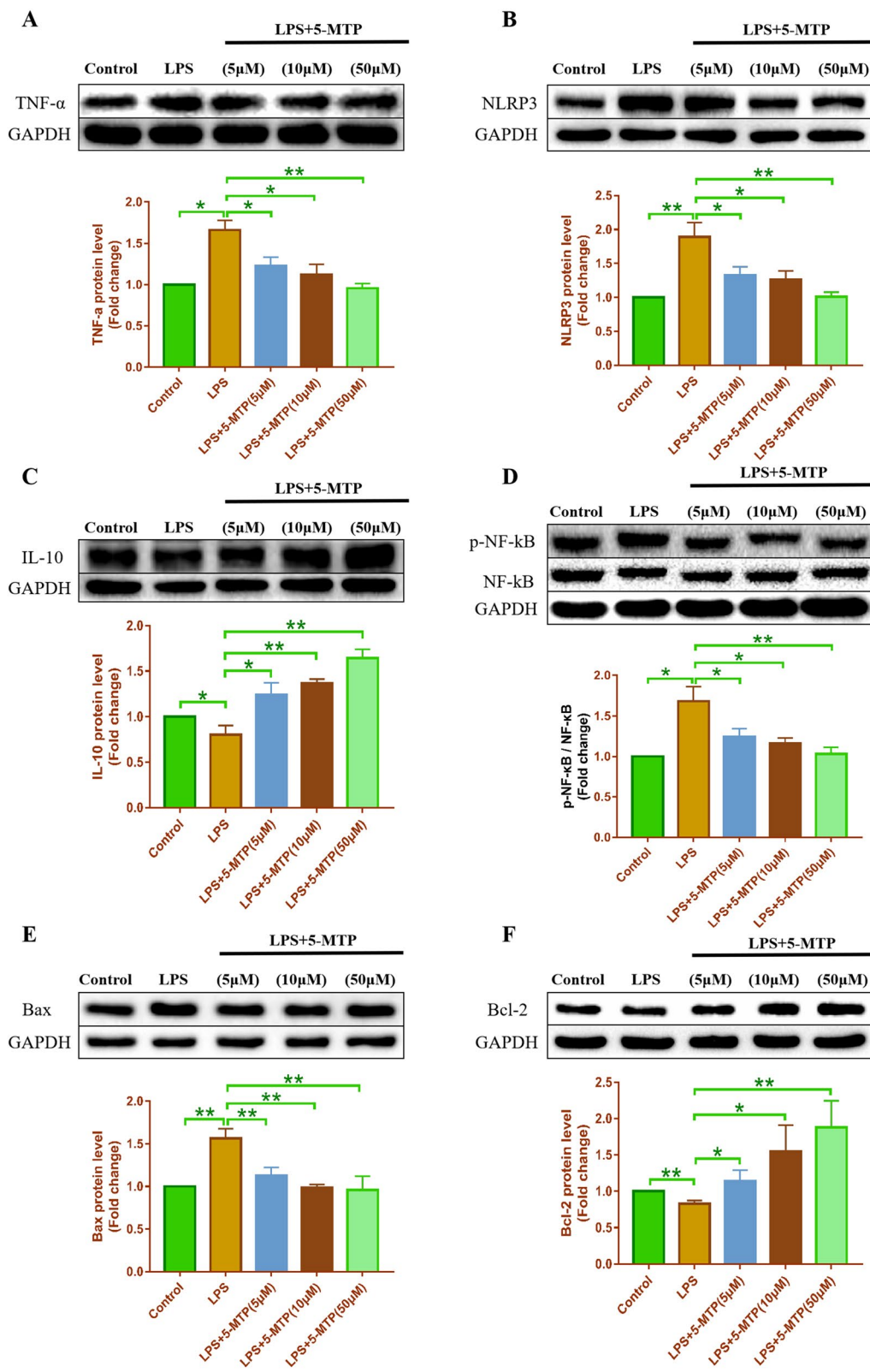


Fig. 6. (See legend on previous page.)

the anti-apoptotic protein level of Bcl-2 was considerably reduced ( $p < 0.05$ ) compared to the control group, and this effect was reversed by pretreatment with 5-MTP (Fig. 6F). The findings indicated that 5-MTP pretreatment could inhibit LPS-induced apoptosis in cultured CFs.

## Discussion

Previous studies have reported that 5-MTP possesses various biological features, like anti-tumorigenesis, vascular protection, anti-inflammation, anti-fibrosis, and anti-senescence [9–12]. 5-MTP acts as a COX-2 suppressor in cancer cells, inhibiting their motility and epithelial-mesenchymal transition [14, 15]. Hydroxyindole-O-methyltransferase (HIOMT) exerts a crucial role in catalyzing the synthesis of 5-MTP. Chen et al. observed that HIOMT-298 is essential for the formation of 5-MTP and that 5-MTP can inhibit A549 cancer cells from proliferation [9]. Recently, 5-MTP has been identified as an essential endothelial factor, which exhibited vasoprotective and anti-inflammation abilities [11]. For example, Chen et al. found that 5-MTP can ameliorate arterial denudation-induced intimal hyperplasia by opposing effects on vascular endothelial and smooth muscle cells [16]. Fang et al. also discovered that 5-MTP, an endogenous tryptophan metabolite, inhibited lung fibrosis by decreasing the PI3K/AKT and TGF- $\beta$ /SMAD3 signaling pathways [10]. In stress-induced mesenchymal stem cell (MSC) senescence, 5-MTP also has anti-senescence properties through upregulating FoxO3a and mTOR [12]. What's more, Hsu et al. further observed a protective effect of 5-MTP on heart of the sprague dawley rats after myocardial infarction via inhibiting oxidative stress and inflammatory response [17]. Therefore, we speculated that 5-MTP might exhibit a protective effect in SIC. The present study was the first time to investigate the role of 5-MTP, a tryptophan metabolite, in LPS-induced cardiac injury and dysfunction. We conducted in vivo and in vitro investigations on LPS-induced heart damage and dysfunction to demonstrate 5-MTP's putative cardioprotective properties. The findings revealed that 5-MTP pretreatment helped to prevent heart damage and dysfunction caused by LPS by reducing inflammation and apoptosis, with the NF- $\kappa$ B signaling pathway playing a role.

In heart tissues and cultured CFs, 5-MTP pretreatment decreased inflammation produced by LPS. Though the SIC pathological changes persist unclearly, inflammation is well known as a critical contributor to the development and progression of SIC [18, 19]. 5-MTP pretreatment significantly reduced LPS-induced cardiac inflammation, as indicated by reduced levels of pro-inflammatory cytokines (TNF- $\alpha$ , IL-6, and IL-1 $\beta$ ) in serums, heart

tissues and cultured CFs. The anti-inflammatory cytokine IL-10 is essential for inflammation development [20]. And it increased markedly after 5-MTP pretreatment in the present study. NF- $\kappa$ B signaling pathway is involved in the production of pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, and TNF- $\alpha$  [21]. As a result, we investigated the effect of 5-MTP pretreatment on NF- $\kappa$ B signaling pathway activation. According to the findings of this study, LPS increased the phosphorylated (p)-NF- $\kappa$ B protein expression level in heart tissues and cultured CFs. At the same time, this effect after 5-MTP pretreatment was significantly reduced. These data suggested that pretreatment with 5-MTP inhibited the NF- $\kappa$ B signaling pathway, hence reducing LPS-induced myocardial inflammation.

In heart tissues and cultured CFs, 5-MTP pretreatment reduced apoptosis induced by LPS. Apoptosis has also been implicated in the pathological changes associated with SIC [13]. Numerous investigations have discovered that LPS-induced inflammation may promote apoptosis [22, 23]. Anti-apoptotic (Bcl-2) and Pro-apoptotic (Bax) proteins balance have a significant effect on myocardial apoptosis induced by LPS [24]. The protein level of Bax increases in LPS-induced cardiac injury and dysfunction, while inhibition of Bax reduces cardiac apoptosis to alleviate cardiac damage and dysfunction induced by LPS [25]. In contrast, after LPS stimulation, the protein level of Bcl-2 decreases in heart tissues, whereas an increase in Bcl-2 attenuates cardiac injury [26]. Therefore, the Bax and Bcl-2 protein level changes will aggravate cardiac injury and dysfunction induced by LPS, while relieving these changes might alleviate LPS-induced cardiac damage. Besides, the proteins of cleaved-Caspase-1 and cleaved-Caspase-3 also play a vital part in developing cardiac apoptosis [27]. In the current study, LPS triggered myocardial apoptosis, as evidenced by elevations in cleaved-Caspase-1, cleaved-Caspase-3, Bax protein levels, and decreased Bcl-2 protein level. At the same time, 5-MTP pretreatment significantly reversed this effect. These findings revealed that 5-MTP pretreatment could alleviate LPS-induced cardiac apoptosis.

The present study has some limitations. Above all, the molecular mechanisms involved in the present study need further in-depth investigations. Besides, A variety of experimental methods are also needed to verify the reliability of the results in the present study.

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## Author contributions

The present study was designed and funded by Professor Y-fD. YF completed the study and prepared the manuscript. Both authors read and approved the final manuscript.

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# Availability of data and materials

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

# Declarations

# Competing interests

The authors declared that they have no conflict of interest in the present study.

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