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# Chinese Herbal Formula Zhuangtongyin Decoction Protects against Myocardial Ischemia by Inhibiting the NF-κB/NLRP3 Inflammasome Pathway

**Meiye Song[1], Hanqing Tang[1*], Keming Li[1], Jiahui Li[1], Jing Zhou[1], Songyi Mo[1], Lingling Huang[1], Caiyan Yang[2], Wenchong Wang[1]**

[1]Basic Medical College of Youjiang Medical University for Nationalities, Baise, China
[2]Guangxi Database Construction and Application Engineering Research Center for Intracorporal Pharmacochemistry of TCM, Youjiang Medical University for Nationalities, Baise, China
Email: 410525595@qq.com, *phdtanghanqing@163.com, lkming@ymun.edu.cn, 121458@ymun.edu.cn, Zjing@ymun.edu.cn, 00433@ymun.edu.cn, Lingling_h@ymun.edu.cn, ycyll@ymun.edu.cn, wangwenchong0924@126.com

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## Abstract

**Background:** The medical community has been trying to reduce the high mortality rate of cardiovascular diseases and find effective treatments; however, the results have not been satisfactory. Zhuangtongyin (ZTY), a Chinese herbal formula, has received significant attention owing to its efficacy in the treatment of myocardial ischemia. However, there are few reports on the mechanisms of its action, which hinder its widespread dissemination. Recent studies have shown that the effects of ZTY are associated with the NF-κB/NLRP3 inflammasome pathway. In this study, we investigated the underlying mechanisms of ZTY in myocardial ischemia through the NF-κB/NLRP3 inflammasome pathway. **Methods:** A myocardial ischemia model was established by ligating rats' left anterior descending coronary artery. The rats were randomly divided into five groups: control group, model group, sham operation group, ZTY group (13.6 g/kg), and NF-κB group (0.12 g/kg). All groups, except the control group, received oral administration for 28 days. The NF-κB group received peritoneal injection of PDTC for 28 days. ECG, assessment of myocardial ischemia injury, histological examination, and analysis of biochemical indices of myocardial tissue were performed to evaluate myocardial injury and drug protection. Inflammation levels were assessed by Masson staining and Elisa, and expression of transforming growth factor (TGF-β1), α-smooth muscle actin (α-SMA), and connexin 43 (Cx43) was detected by immunohistochemistry. **Results:** Compared to the control group, the model group showed

obvious abnormalities in myocardial tissue and increased levels of inflammatory cytokines. ZTY therapy inhibited the increases in lactate dehydrogenase (LDH), creatinine kinase (CK), creatinine kinase isoenzyme (CK-MB), tumour necrosis factor $\alpha$ (TNF-$\alpha$), and interleukin-6 (IL-6). The immunohistochemical results showed that ZTY treatment inhibited TGF-$\beta$1 expression, suggesting that the anti-inflammatory effect of ZTY was achieved by inhibiting NF-$\kappa$B signalling. **Conclusion:** ZTY can effectively alleviate myocardial injury, and its protective effect against myocardial injury is related to inhibition of the NF-$\kappa$B/NLRP3 inflammasome pathway.

## Keywords

Zhuangtongyin, Myocardial Ischemia, Inflammation, NF-$\kappa$B, NLRP3

## 1. Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide. Such diseases not only pose a threat to the lives of patients, but also impose tremendous strain on the health system. In recent years, with the improvement in living conditions and aging of the world population, the number of deaths from cardiovascular diseases has been higher and younger than ever before. Ischemic heart disease accounts for 16% of the deaths worldwide [1]-[3]. Myocardial ischemia-induced injury is quite common in clinics and can be divided into chronic myocardial ischemia and acute myocardial ischemia. It is mainly caused by coronary artery obstruction, hypertension, hyperlipemia, insufficiency of blood supply, myocardial atrophy, myocardial anoxia, and other causes, and mainly manifests as chest pain, dyspnoea, and arrhythmia [4]. If left untreated, it usually develops into angina pectoris and heart failure. This may lead to myocardial infarction or sudden death [5]. Both types of myocardial ischemia require active treatment and management to prevent their complications and exacerbations.

Currently, the main interventions for myocardial ischemia are routine medication and surgery. Although some Western medicines take effect quickly and have specific therapeutic effects on some serious symptoms, long-term use produces drug resistance and serious side effects, such as anticoagulants, which may cause bleeding, and receptor blockers, which may cause hypotension [6] [7]. Many drugs have a single target, which limits their clinical use (e.g. statins, thrombolytics, calcium channel blockers, and ADP receptor antagonists) [8]. Interventional surgery is often associated with expensive surgery, whereas bypass surgery is traumatic and high-risk. Therefore, finding safe medicines from plants that can effectively improve myocardial ischemia without causing side effects is necessary. The culture of traditional Chinese medicine (national medicine) has a long history; for some famous doctors, ancient prescriptions are still being tested. This is because it not only focuses on the disease itself but also on conditioning the internal environment of the human body and promoting physical and mental health. In today's

globalised world, traditional Chinese medicine, with its natural, mild, and comprehensive characteristics, provides a new perspective and method for modern medicine. Compared with Western medicine, it has a low cost, is a safe, natural plant, can integrally condition the internal organs of the body, and can be consumed for a long time. However, basic research still needs to be stronger and needs to be strengthened. Recent studies have shown that the NF-κB/NLRP3 signalling pathway is closely related to the inflammatory response. However, there have been no reports on whether ZTY affects this pathway. We aim to verify this.

Research has verified that Myocardial ischemia induces a systemic inflammatory response and promotes production of TNF-α, IL-6, and other inflammatory factors. The NF-κB/NLRP3 signalling pathway is also involved in mediating the inflammatory response in rats with myocardial ischemia [9] [10]. The NF-κB/NLRP3 pathway is an important cell signalling pathway that plays a key role in various biological processes, including the inflammatory response, apoptosis, cell proliferation, and tumourigenesis [11]. The NF-κB/NLRP3 pathway can upregulate the expression of inflammatory genes, including inflammatory cytokines, such as TNF-α and IL-6. In cardiomyocytes, ZTY inhibits inflammation by downregulating the expression of TNF-α and IL-6 and decreasing the activation of the NF-κB pathway. The NF-κB/NLRP3 signalling pathway has not been sufficiently studied in myocardial ischemia. For example, it has little effect on myocardial damage and cardiomyocyte protein expression. Studies have shown that myocardial ischemia and hypoxia increase reactive oxygen species (ROS) levels. Meanwhile, ROS acts as an upstream signal, activates NF-κB through phosphorylation, upregulates NLRP3 inflammatory bodies, promotes TNF-α secretion, and amplifies inflammatory responses [12] [13]. Promote the transcription and expression of downstream inflammation-related genes, such as COX-2 and iNOS, and increase the levels of inflammatory cytokines TNF-α, IL-6, and TGF-β1, wherein IL-6 leads to the enhancement of angiogenesis and an increase in vascular permeability, which are pathological features of inflammatory lesions [14] [15]. TNF-α can enhance phagocytosis and cause inflammatory cells to rapidly release large amounts of oxygen-free radicals [16] [17]. TGF-β1 regulates cell proliferation, differentiation, and extracellular matrix production [18] [19]. These inflammatory factors can activate immune cells, induce inflammatory reactions, and promote the aggregation and adherence of inflammatory cells, thus aggravating cardiovascular damage [20].

The NF-κB/NLRP3 pathway may also affect the survival of cardiomyocytes by promoting apoptosis, which may lead to increased release of myocardial enzymes. Creatinine kinase (CK), creatinine kinase isoenzyme (CK-MB), lactate dehydrogenase (LDH), and troponin I (cTnI) are the main markers used to assess myocardial injury. Among them, cTnI exists only in cardiomyocytes, but because it can be secreted into the blood at the early stage of myocardial injury, it has great specificity, so cTnI is one of the "gold standards" for judging myocardial injury with CK-MB [21] [22]. α-SMA transfers force between adjacent cardiomyocytes;

therefore, abnormal $\alpha$-SMA can cause cardiomyocyte injury by causing persistent stress in cardiomyocytes [23] [24]. Cx43 mediates intercellular communication, and myocardial ischemia can impair the secretion of Cx43 in extracellular vesicles derived from cardiomyocytes [25] [26]. In some cases, activation of the NF-$\kappa$B/NLRP3 pathway may also directly or indirectly affect the expression and release of myocardial enzymes such as LDH and cTnI.

ZTY is composed of three special Chinese Zhuang nationality medicinal material herbs of fortune: euonymus, tianqi, and polygala fallax hemsl. Euonymus fu fang vine, according to the records in "Medicine Property Test", has the function of "promoting qi and promoting blood circulation"; Tianqi, according to the "new compilation of herbal medicine" records, with "hemostasis and tonify deficiency" role, polygala fallax hemsl according to the records in "Guangxi province herbal medicine" has the function of "Tonifying qi and blood, strong bones and muscles". Therefore, this prescription can alleviate ischemia and hypoxia. We screened the proportions and doses of the drugs using a literature search and pre-experiments [27] [28]. As the basic research on ZTY for protecting the myocardium is currently lacking, the present research aims to establish a myocardial ischemia rat model, and the level of myocardial zymogram and inflammatory factors in the serum of rats was detected by taking ZTY as an intervention drug. To observe and study whether ZTY can inhibit inflammation through the NF-$\kappa$B/NLRP3 route, verify the protective effect of ZTY on rat myocardium, and provide a new effective drug for the clinical treatment of coronary heart disease and myocardial ischemia.

## 2. Materials and Methods

### 2.1 Materials

The enzyme-linked immunosorbent assay (ELISA) kits for IL-6, TNF-$\alpha$, cTnI, CRP, and IFN-$\gamma$ were purchased from Elabscience Biotechnology Co. Ltd. (Wuhan, China). Blood biochemical kits for CK, CK-MB, and LDH were purchased from Rayto Life Science Co., Ltd. (Shenzhen, China). All antibodies used for immunohistochemistry were purchased from Biosynthesis Biotechnology Co. Ltd. (Beijing, China). The Masson staining kit was purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). The TTC staining kit was purchased from Leagene Biotechnology Co. Ltd. (Beijing, China).

### 2.2. Plant Material and Extraction

Fortune euonymus, tian qi, and polygala fallax hemsl were purchased from the Pharmacy of Jiexi Yi bai Pharmacy Co., Ltd. (Guangzhou, China) and were identified by Associate Professor Ke-Ming Li, Basic Medical College of Youjiang Medical University for Nationalities. Danshen Dripping Pills were purchased from Tasly Pharmaceutical Co., Ltd. (Tianjin, China).

According to the internal dosage of Zhuangtong Yin for normal adults (body weight: 60 kg), namely 30 g of fortune euonymus, 20 g of polygala fallax hemsl,

and 15 g of tian qi, the mass ratio of each drug for the preparation of ZTY water-decocted liquid was determined. That is, the ZTY decoction pieces are weighed according to the drug mass ratio of fortune euonymus: polygala fallax hemsl: tian qi = 6:4:3. Then, tian qi and polygala fallax hemsl were added to an appropriate amount of distilled water and soaked for half an hour, boiled for 30 min, and then fortune euonymus was added; the mixture was boiled for a further 30 min, cooled to room temperature, and filtered through gauze. The extracts were filtered and used in animal studies.

### 2.3. Experimental Animals

Fifty Sprague-Dawley rats (Rattus norvegicus, half male and half female) with a body mass of 260 ± 20 g were purchased from the Beijing Vito Lihua Laboratory Animal Technology Co., Ltd. (Certificate number of experimental animals, SCXK2023-0014). The rats were kept at the SPF-grade Animal Experiment Centre of Youjiang Medical University for Nationalities, and the animals had free access to water and a chow diet during the experiments. The temperature of the feeding environment was maintained at 25˚C ± 2˚C and the proper temperature. The handling and rearing of animals in the experiment conformed to ethical standards. The animals fasted for 12 h before the experiment and during the sample collection period but received water. The animal experiments were approved by the Committee of Experimental Animal Ethics of Youjiang Medical University for Nationalities (approval No. 2023050701). Throughout the experiments, the animals were handled according to the requirements and guidelines issued by the Ministry of Science and Technology, China.

Fifty rats were randomly divided into five groups (n = 10): control, model, sham operation (threading without ligation), ZTY (13.6 g/kg) (this dose was selected based on the consideration that the medium-dose group (13.6 g/kg) had the best effect in the previous experiments of our research group), and NF-κB (PDTC 0.12 g/kg) group [29]. Weighed was recorded and fixed on the operating table after anaesthesia. A longitudinal incision was made at the left apical pulsation, the muscles were bluntly separated, the third rib and intercostal muscles were snipped, and the thoracic cavity was exposed using a retractor to ensure stability of the operation above the heart [30] [31]. Ligation was performed 3 mm below the left anterior descending coronary artery (LAD) using a 6-0 needle suture. The left ventricular wall changed from red to white, indicating that ischemia occurred. The retractor was disconnected until spontaneous breathing was observed. The rat muscles were aligned and sutured. Compared to that before ligation, the ligation part changed from red to white. If the ST-segment elevation of lead II was >0.02 mV, the model was successfully constructed [32]. Three days after the operation, the rats were intragastrically administered once a day when their condition was stable. Rats in the sham operation group were administered an equal volume of saline, and rats in the NF-κB group were injected intraperitoneally with PDTC. Finally, the rats were sacrificed under excessive anaesthesia to minimise pain.

### 2.4. ECG Detection

Electrocardiography (ECG) monitoring was performed using a three-channel electrocardiogram recorder. Each rat was placed on an operating table, each lead was clamped to its limbs, and an electrocardiogram was printed after the waveform was stabilised.

### 2.5. Heart Function Examinations

Two hours after the last dose, the left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), stroke volume (SV), and left ventricular ejection fraction (LVEF) of rats in each group were measured using a colour Doppler ultrasound diagnostic instrument (probe frequency: 2.5 MHz). Data from at least three cardiac cycles were observed and recorded, and the average value was used as the test result.

### 2.6. Samples Collection

3weeks after administration, blood was collected from the abdominal aorta, left at room temperature for 30 min, centrifuged at 3500 × g for 15 min, and the supernatant was collected and stored at −80˚C. The heart was removed immediately after blood collection and perfused with pre-cooled saline to remove the intraventricular blood. The rats were sacrificed and myocardial tissue was collected for further analysis.

### 2.7. Myocardial Specific Enzyme Assays

Whole blood from rats was centrifuged at 3000 r·min$^{-1}$ at 4˚C for 15 min, and the supernatant was collected and stored at −80˚C for detection. The CK, CK-MB, and LDH activities were determined using the Creatine Kinase Assay Kit, Creatine kinase isoenzyme Assay Kit, and Lactate dehydrogenase Assay Kit (Radito Life Science Co., Ltd., Shenzhen, China) and were performed according to the instructions and the experiment was repeated 3 times.

### 2.8. Determination of Inflammatory Cytokines in Serum

Blood was collected from the rat abdominal aorta and left to stand at room temperature for 20 min. The supernatant was collected after centrifugation at 3000 r·min$^{-1}$ for 15 min at 4˚C. The rat serum was tested for inflammatory factors (TNF-$\alpha$, IL-6, cTnI, CRP, and IFN-$\gamma$) according to the manufacturer's instructions (Elabscience Biotechnology Co., Ltd., Wuhan, China), and the experiment was repeated three times.

### 2.9. TTC Staining

Rat hearts were rinsed with PBS buffer. Ventricular tissues were cut into equal thicknesses. The fragments were then added to 2% TTC (Beijing League Biotech Co. Ltd. Beijing, China.) solution and incubated at 37˚C for 15 min. Following

incubation, myocardial tissue myocardial ischemia was pale compared to normal myocardial tissue.

### 2.10. Masson Staining

The tissue was perfused with pre-cooled saline until no blood was spilled, and the myocardial tissue was immobilized in 4% paraformaldehyde, embedded in paraffin, sectioned (4μm). Masson staining according to the kit instructions (Solarbio Science & Technology Co., Ltd., Beijing, China) and the experiment was repeated 3 times. Histopathological changes in the sections were examined under a microscope (Nikon E100, Tokyo, Japan) and photographed at 200× magnification.

### 2.11. Immunohistochemically Staining

The myocardial tissue was fixed with 4% polyformaldehyde for 24 h and embedded in paraffin. Dewax the paraffin sections and incubate in 3% hydrogen peroxide at room temperature for 25 minutes in the dark, then with $\alpha$-SMA (1: 200), TGF$\beta$1 (1:500), and Cx43 (1: 500). The antibodies were incubated overnight at 4˚C. The slides were incubated with biotinylated secondary antibody for 50 min, developed with avidin peroxidase and DAB, and re-stained with haematoxylin. A permanent mounting medium was applied to the slides, which were then covered with cover glass. Positive cells appear in brown regions. After immunohistochemical staining, myocardial tissue sections were observed under a microscope, and positive cells were quantitatively analyzed. The staining intensity of positive cells was evaluated by Image J to quantify the expression levels of TGF-$\beta$1, $\alpha$-SMA and Cx43 in each group.

### 2.12. Statistical Analysis

Statistical analysis was performed using SPSS26.0 and Graph Prism9.0. One-way analysis of variance was used to evaluate the results, followed by the Student-Newman-Keuls test to compare different groups. Differences were considered statistically significant at $P < 0.05$.

## 3. Results

### 3.1. ECG Manifestations of Each Group

In this study, ECGs were recorded seven days after dosing, and the results are shown in **Figure 1(A)**. As shown in **Figure 1(A)**, the electrocardiogram (ECG) recordings indicated significant changes in the ST segment among the different groups. The model group exhibited a marked elevation in the ST segment compared to the control group, indicating the presence of myocardial ischemia. However, treatment with ZTY and PDTC (NF-$\kappa$B group) resulted in a notable reduction in ST segment elevation, suggesting that both interventions were effective in alleviating ischemic conditions. Statistical analysis revealed that the differences between the model group and the treatment groups were significant ($P < 0.05$), confirming the efficacy of ZTY in protecting myocardial function.

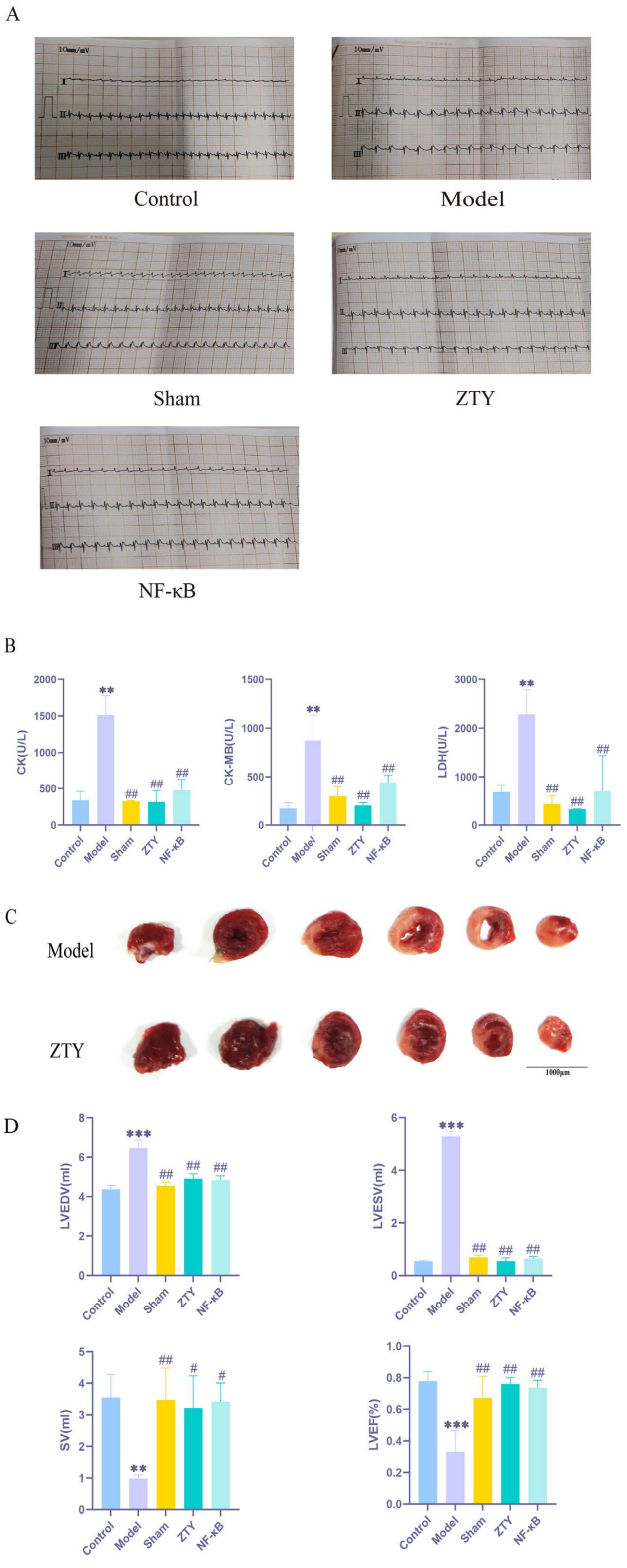


**Figure 1.** Protective effect of ZTY on myocardial ischemia. (A) ECG; (B) Serum CK, CK-MB and LDH levels; (C) Myocardial ischemia image determined by TTC staining. (Red is the normal area, white is the ischemic area); (D) The results of the cardiac function test in each group. Data are Mean ± SD (n = 10 per group) $^{**}P < 0.01$ versus control, $^{\#}P < 0.05$ versus model, $^{\#\#}P < 0.01$ versus model.

### 3.2. Myocardial Enzyme Levels by Blood Biochemistry

These results show that ZTY had a positive effect on myocardial ischemia. Figure 1(B) illustrates the serum levels of myocardial enzymes, including CK, CK-MB, and LDH. The model group showed significantly elevated levels of these enzymes, indicative of myocardial injury. In contrast, the ZTY group exhibited a marked decrease in enzyme levels, demonstrating the protective effect of ZTY against myocardial injury. The NF-$\kappa$B group also showed significant reductions in enzyme levels, further supporting the conclusion that ZTY and NF-$\kappa$B inhibition contribute to myocardial protection ($P < 0.05$). The myocardial ischemic area was also reduced in the ZTY group compared with that in the model group (Figure 1(C)).

### 3.3. Cardiac Function by Echocardiography

Echocardiographic measurements depicted in Figure 1(D) reveal significant differences in cardiac function parameters among the groups. The model group had elevated LVEDV and LVESV, leading to reduced stroke volume and LVEF. Conversely, the ZTY group demonstrated improved cardiac function with decreased LVEDV and LVESV, resulting in increased SV and LVEF. The NF-$\kappa$B group showed similar improvements, indicating the effectiveness of both treatments in enhancing cardiac function ($P < 0.05$).

### 3.4. Expression of TNF-$\alpha$, IL-6, cTnI, CRP, and IFN-$\gamma$ Levels by ELISA

To further evaluate and verify the protective effect of ZTY, we measured the serum levels of TNF-$\alpha$, IL-6, cTnI (Figures 2(A)-(C)), CRP (Figure 3(A)), and IFN-$\gamma$ (Figure 3(B)). TNF-$\alpha$, IL-6, cTnI, CRP, and IFN-$\gamma$ levels were significantly increased in the model group. Notably, the ZTY group could inhibit the increase in inflammatory cytokine secretion in the serum, and the anti-inflammatory effect was better ($P < 0.05$).

### 3.5. Myocardial Morphological Changes were Detected by Masson Staining

Masson staining test showed that the myocardial structure was normal, the muscle fiber was regular, and the degree of myocardial fibrosis was significantly reduced compared with the model group. (Figure 2(D)). Compared to the control group, the model group showed an increased degree of myocardial fibrosis and inflammatory cell infiltration ($P < 0.05$). Myocardial injury in the ZTY and NF-$\kappa$B groups was lower than that in the Model group ($P < 0.05$). The histopathological results confirmed that ZTY alleviated myocardial ischemic injury.

### 3.6. Expression of TGF-$\beta$1, $\alpha$-SMA, and Cx43 at the Protein Level by Immunohistochemistry

As shown in Figure 2(E), Figure 2(F), compared with the control group, the positive expression level of TGF-$\beta$1 in the model group was higher, and the shape was irregular. However, the positive expression was reduced in the other groups

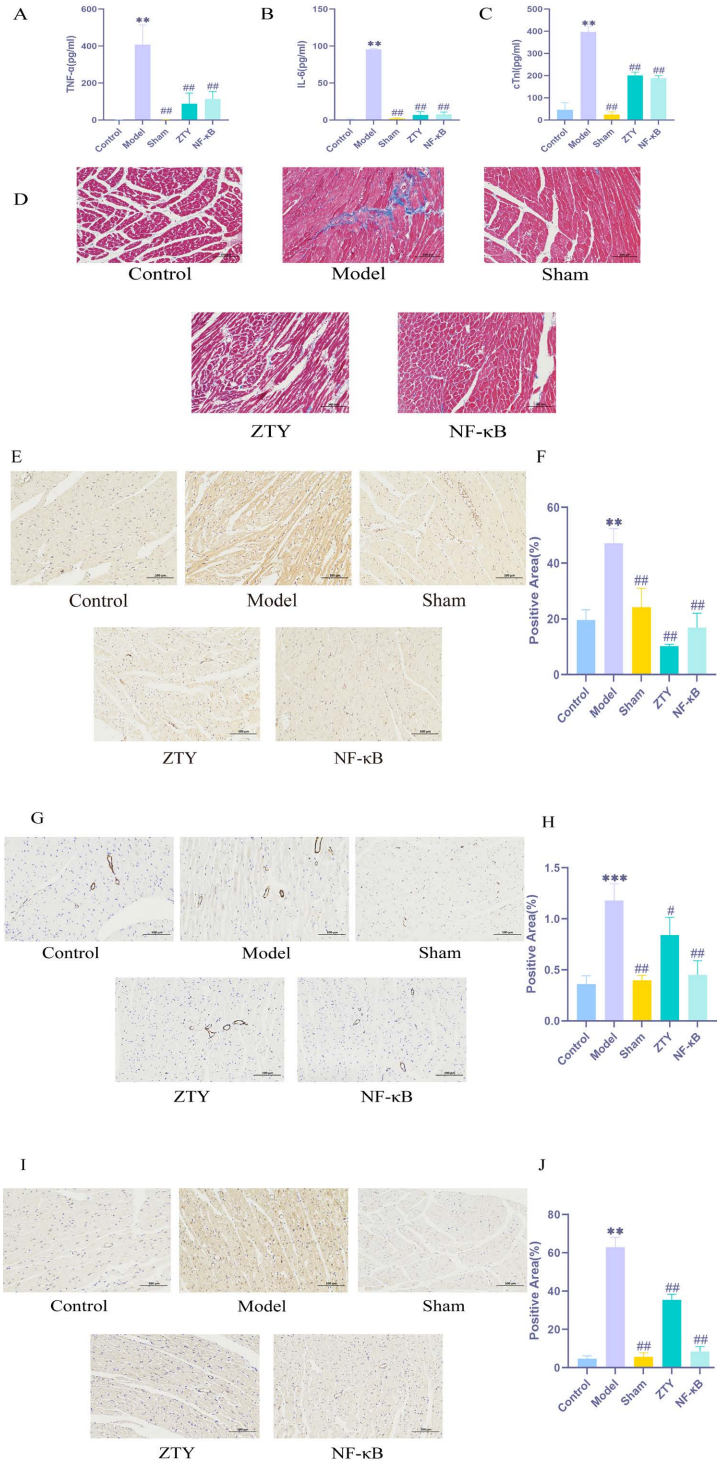


**Figure 2.** The expression of ZTY on myocardial ischemia and Immunohistochemistry of myocardial tissue (200× magnification). (A) Serum TNF-$\alpha$ levels; (B) Serum IL-6 levels; (C) Serum cTnI level. Compared with the control group, $^{**}P < 0.01$, compared with the model group, $^{\#\#}P < 0.01$; (D) Myocardial damage was assessed by Masson staining, scale bar, 100 μm; (E) (F) Immunohistochemistry and positive expression rate of TGF$\beta$1; (G) (H) Immunohistochemistry and positive expression rate of $\alpha$-SMA; (I) (J) Immunohistochemistry and positive expression rate of Cx43. All data are shown as mean ± SEM. $^{**}P < 0.01$ versus control, $^{\#}P < 0.05$, compared with the control group, $^{\#\#}P < 0.01$ versus model.

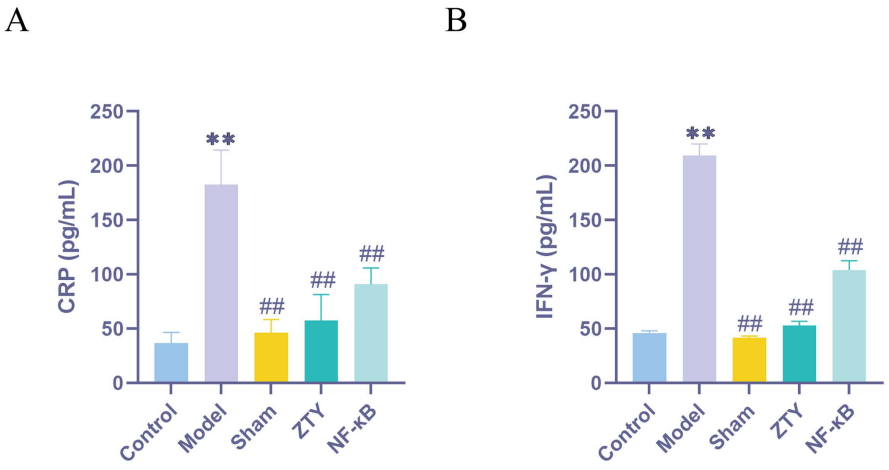


**Figure 3.** Expression of ZTY in inflammation. (A) CRP level in serum; (B) The level of IFN-$\gamma$ in serum. All data are shown as mean ± SEM. $^{**}P < 0.01$ versus control, $^{\#\#}P < 0.01$ versus model.

compared to that in the model group. The results of $\alpha$-SMA expression showed that the positive expression level of the model group was higher than that of the control group, and the positive expression level of the other groups was lower than that of the model group (**Figures 2(F)-(H)**). As shown in **Figure 2(I)**, **Figure 2(J)**, Cx43 expression increased in the model group, and ZTY intervention reversed the increase in Cx43 expression. Therefore, the results showed that ZTY may inhibit the NF-$\kappa$B pathway by downregulating the expression of TGF-$\beta$1/$\alpha$-SMA/Cx43. These results suggest that ZTY effectively downregulates the expression of these proteins, which are associated with inflammation and myocardial remodeling ($P < 0.05$).

## 4. Discussion

This study focused on myocardial ischemia, a common cardiovascular disease that threatens both life and health. Owing to the limitations of the current treatment methods and the fact that ZTY is widely used in clinical practice, there is a need for more basic research on myocardial protection. In this study, different experimental groups were established using a rat model of myocardial ischemia. Various detection methods have been used to study cardiac function, myocardial enzymes, inflammatory factor levels, and myocardial histomorphology. The specific mechanisms of action of ZTY in treating myocardial ischemia were clarified, and its possible mechanism of action was discussed, which reflected the combination of traditional Chinese medicine and modern medicine, and helped promote the modernization of traditional medicine. According to previous studies, a decrease in cardiac oxygen supply caused by myocardial ischemia can lead to abnormal myocardial energy metabolism and cannot support normal cardiac activity [33] [34]. The progression of the disease, whether acute or chronic, is closely related to the development of inflammation. Therefore, the relief of myocardial ischemia symptoms requires a deeper understanding of the underlying mechanisms,

especially the involvement of the inflammatory pathways. The NOD-like receptor thermal protein domain-associated protein 3 (NLRP3) inflammasome is involved in the pathological processes of many diseases and has been shown to be relevant to myocardial ischemia [35] [36]. Notably, the traditional Chinese medicine formula ZTY can significantly downregulate the NF-$\kappa$B/NLRP3 signalling axis and reduce the inflammatory response to exert therapeutic effects on myocardial ischemia.

ZTY is still a safe method for the clinical treatment of myocardial ischemia, and studies have shown that ZTY can effectively reduce proinflammatory factors, such as TNF-$\alpha$ and IL-6, in patients, which can lead to the disappearance of inflammation within a short period [37]. The neuroprotective effects of ZTY may be partly mediated by the inhibition of inflammation and microglial activation. ZTY therapy not only causes inflammation to disappear quickly, but most importantly, it can also prevent recurrence of the condition from the root. ZTY is a specific therapeutic strategy in Zhuang nationality medicine that has been widely used in Guangxi Zhuang, an autonomous region in China. ZTY contributes to regulating qi and blood circulation, tonifying deficiency, and ultimately brings the body into a healthy synchronisation and harmonious state. However, existing studies have only explored the changes in the expression of inflammation-related factors [38]. Our study explored the specific mechanisms and possible molecular mechanisms of ZTY regulation of inflammatory factors to improve myocardial ischemia, enrich the current research content of ZTY, and conduct in-depth exploration to a certain extent.

Elevated levels of inflammatory cytokines, such as TNF-$\alpha$, IL-6, cTnI, CRP, IFN-$\gamma$, and myocardial enzymes, such as CK, CK-MB, and LDH, characterise myocardial ischemic injury [39]-[42]. In our present experimental work, it was observed that the ST-segment elevation of the rats in the ZTY group and NF-$\kappa$B group were decreased relative to those in the model group; and the levels of TNF-$\alpha$ and inflammatory factors IL-6, CRP, and IFN-$\gamma$ levels were also decreased, suggesting that ZTY therapy acts as a similar NF-$\kappa$B inhibitor, further validating the reports related to the involvement of ZTY therapy in regulating the levels of related inflammatory factors. Compared to the model group, the ZTY and NF-$\kappa$B groups had decreased serum CK, CK-MB, LDH, TNF-$\alpha$, IL-6, and cTnI levels and decreased myocardial ischemia and inflammatory factor infiltration. This result provides strong evidence that ZTY alleviates the inflammatory response in myocardial ischemia, and that this effect may be related to the inhibition of NF-$\kappa$B expression.

TNF-$\alpha$ and IL-6 activate the NLRP3 inflammasome by activating the NF-$\kappa$B signalling pathway. Once activated, the NLRP3 inflammasome facilitates the maturation and release of proinflammatory cytokines such as IL-1$\beta$ and IL-18. These cytokines amplify the immune responses and trigger inflammation. This interaction forms a positive feedback loop that exacerbates inflammation during myocardial ischemia [43]. Recent studies have shown that the inhibition of the NF-

κB/NLRP3 signalling pathway can ameliorate myocardial ischemia-reperfusion injury, confirming the interconnectivity and critical roles of TNF-α, IL-6, and NLRP3 in myocardial ischemia. Moreover, it has been found that certain drugs or compounds, such as scutellarin and dexmedetomidine, can mitigate myocardial ischemia-reperfusion injury by suppressing the NF-κB/NLRP3 signalling pathway. These findings provide novel strategies for the treatment of myocardial ischemia.

Therefore, TNF-α, IL-6, and NF-κB/NLRP3 play distinct roles in myocardial ischemia and interact with one another, collectively driving the development of the inflammatory response and exacerbation of myocardial damage. Understanding these interactions is important for the development of new therapeutic approaches to myocardial ischemia.

Regarding its translational potential, model evidence is both promising and suggestive of its possible efficacy in humans. In animal models of chronic myocardial ischemia, ZTY has been shown to have a positive effect on cardiac function and structure. For example, it may improve myocardial contractility and reduce the area of myocardial infarction. This indicates that ZTY could potentially have a similar beneficial effect on human patients with chronic myocardial ischemia. Moreover, its action on specific pathways related to myocardial protection gives it an advantage over nonspecific drugs, potentially leading to more targeted and effective treatments with fewer off-target effects in clinical trials.

However, there were some safety concerns. In animal studies, abnormal electrocardiogram (ECG) changes have been observed after ZTY treatment, which could be an indication of potential cardiac arrhythmia in humans. Additionally, the potential for interference with normal cardiac conduction systems, which might be a consequence of its effectiveness in modulating cardiac-related mechanisms, is another factor that requires further examination.

The incorporation of a broad range of biomarkers relevant to myocardial ischemia is essential. By doing so, we can obtain a more comprehensive understanding of ZTY's systemic effects. For example, not only should we focus on traditional biomarkers, such as cardiac troponin and CK-MB, but also on emerging biomarkers such as high-sensitivity C-reactive protein (hs-CRP) and growth-differentiation factor-15 (GDF-15). This will help better predict the overall impact of ZTY on the heart, including both its desired effects on myocardial protection and any potential adverse effects. Regulatory challenges also pose great difficulty in the context of ZTY. As with any new drug for chronic myocardial ischemia, meeting regulatory requirements for safety and efficacy is a complex and time-consuming process. The need to prove the safety of the drug in the face of potential side effects, while also demonstrating its effectiveness based on a comprehensive set of biomarkers related to myocardial ischemia, is a major obstacle in the translation of ZTY from animal studies to successful human trials.

Although this study achieved some results in exploring the improvement effect of ZTY on myocardial ischemia, it also has some limitations. Although it has been

mentioned before that multiple biomarkers should be included, the currently used biomarkers may still not fully reflect all the effects of ZTY on chronic myocardial ischemia in rats. For example, although biomarkers such as LDH in rats and cTnI and CK-MB in rats are concerning, there may be other undiscovered biomarkers related to myocardial ischemia in rats and the action of ZTY.

## 5. Conclusion

In summary, the NF-κB/NLRP3 signaling axis is associated with the onset of myocardial ischemia. ZTY can alleviate the inflammatory response to myocardial ischemia via the NF-κB/NLRP3 signaling axis. This study provides a theoretical basis for the treatment of myocardial ischemia by using ZTY.

## Credit Authorship Contribution Statement

Meiye Song: Writing—original draft, Investigation. Keming Li: Methodology and Investigation. Wenchong Wang: Investigation. Songyi Mo: Methodology. Jiahui Li: Methodology. Jing Zhou: Methodology. Caiyan Yang: Methodology. Lingling Huang: Writing—review & editing, Methodology. Hanqing Tang: Writing—review& editing, Methodology, Funding acquisition.

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## Animal Ethics

Animal experiments were approved by the Experimental Animal Ethics Committee of Youjiang Medical University for Nationalities (approval No. 2023050701).

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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## Abbreviation

| | |
|---|---|
| ZTY | Zhuangtongyin decoction |
| TGF-$\beta$1 | transforming growth factor-$\beta$1 |
| $\alpha$-SMA | $\alpha$-smooth muscle actin |
| Cx43 | Connexin 43 |
| LDH | lactate dehydrogenase |
| CK | creatinine kinase |
| CK-MB | creatinine kinase isoenzyme |
| TNF-$\alpha$ | tumor necrosis factor $\alpha$ |
| IL-6 | interleukin-6 |
| ROS | reactive oxygen species |
| cTnI | troponin |
| LAD | left anterior descending coronary artery |
| LVEDV | left ventricular end-diastolic volume |
| LVESV | left ventricular end-systolic volume |
| SV | stroke volume |
| LVEF | left ventricular ejection fraction |
| CRP | C-reactive protein |
| IFN-$\gamma$ | Interferon |