*Original Research*

# Hydrogen sulfide mitigates myocardial inflammation by inhibiting nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome activation in diabetic rats

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## Impact statement

Diabetic cardiomyopathy is a serious complication of diabetic patients, accompanied by chronic inflammation. The nucleotide-binding oligomerization domain-like receptor protein (NLRP) 3 inflammasome complex is involved in the progression of the inflammatory response of diabetes, including diabetic cardiomyopathy. Hydrogen sulfide ($H_2S$) is a novel endogenous gas messenger. Several pieces of evidence have exhibited that $H_2S$ exerts anti-oxidant and anti-inflammatory activities against hyperglycemia-induced myocardial injury, but the mechanism remains unclear. The current study indicated that $H_2S$ protected the myocardium against hyperglycemia-induced injury by preventing Thioredoxin-interacting protein (TXNIP)-mediated NLRP3 inflammasome complex activation. The inhibition of TXNIP-mediated NLRP3 inflammasome complex would be an efficient therapy for $H_2S$ treatment in diabetic cardiomyocytes.

## Abstract

Inflammation plays a crucial part in hyperglycemia-induced myocardial damage. Hydrogen sulfide has been found to possess multiple biological activities in previous studies. This study investigated whether hydrogen sulfide conferred cardiac protection against damage in a diabetic rat model by inhibiting nucleotide-binding oligomerization domain-like receptor protein (NLRP) 3 inflammasome activation. Male animals were assigned to control, streptozotocin, streptozotocin + sodium hydrosulfide, and streptozotocin + DL-propargylglycine groups. Animals in the three streptozotocin groups were administrated 55 mg/kg streptozotocin by intraperitoneal injection. Streptozotocin + sodium hydrosulfide and streptozotocin + propargylglycine groups were treated with sodium hydrosulfide (56 μmol/kg) and propargylglycine (40 mg/kg), respectively, for four weeks. Estimation of fasting blood glucose, heart-weight/body-weight, cardiac function, and histopathological analysis, and measurement of myocardial enzymes were done to evaluate the degree of cardiac injury. In order to investigate the redox changes, the levels of total antioxidant capacity, malondialdehyde and lipid peroxidation, and the activities of superoxide dismutase, catalase, and glutathione peroxidase were assessed; the protein expression levels of Thioredoxin and Thioredoxin-interacting protein were measured in myocardial tissue. In addition, inflammatory reactions were assessed by measuring the concentration levels of interleukin-6, tumor necrosis factor-α, interleukin-1β, and interleukin-18 in serum and the expression levels of NLRP3 inflammasome complex-associated proteins in cardiac tissue. In the heart, hyperglycemia significantly induced cardiac dysfunction and injury, redox perturbation, and aggravation of inflammatory reactions. However, except for fasting blood glucose, treatment with sodium hydrosulfide significantly ameliorated these alterations, whereas treatment with propargylglycine further aggravated these alterations. This study highlights the protective properties of hydrogen sulfide against hyperglycemia-induced cardiac injury, and its possible mechanism was shown to involve negative regulation of Thioredoxin-interacting protein-mediated NLRP3 inflammasome activation.

**Keywords:** Hydrogen sulfide, diabetes mellitus, rat, inflammation, oxidative stress, nucleotide-binding oligomerization domain-like receptor protein 3 inflammasome

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## Introduction

Diabetes mellitus is a group of chronic metabolic diseases that causes high blood glucose concentrations and a number of serious complications, such as nervous, renal, retinal, and cardiovascular diseases.[1] Diabetic cardiomyopathy (DCM) is a heart disease caused by diabetes mellitus.[2] DCM, characterized by a decreased diastolic and systolic cardiac function and not associated with hypertension and coronary heart disease, is the salient cause of death in patients with diabetes.[3] One of the main causes of DCM is low-grade chronic inflammation in myocardial tissue.[4]

The nucleotide-binding oligomerization domain-like receptor protein (NLRP) 3 inflammasome is a well-known inflammasome complex, which is considered as an important regulator of inflammatory reaction in diabetes mellitus.[5] The NLRP3 inflammasome is mainly comprised of NLRP3 protein, apoptosis-associated speck-like protein (ASC), and procaspase-1.[6] Stimulation of the NLRP3 inflammasome sparks activation of procaspase-1 and subsequent cleavage of pro-interleukin (IL)-18 and pro-IL-1β results in their activation to pro-inflammatory cytokines.[7] TXNIP is a negative regulatory factor of Thioredoxin, which combines with Thioredoxin and inhibits the redox-regulatory activity of Thioredoxin.[8] The expression level of TXNIP protein is powerfully stimulated by extracellular high glucose level, which is now considered as a crucial activator of NLRP3 inflammasome *in vivo*.[9] Many reports have stated that inhibition of NLRP3 inflammasome significantly alleviates DCM.[10,11]

$H_2S$ is a novel gas signaling molecule in mammals and can be produced from L-cysteine by several endogenous enzymes, including cystathionine-γ-lyase (CSE), 3-mercaptopyruvate sulfurtransferase, and cystathionine-β-synthase.[12] Sodium hydrosulfide (NaHS) often serves as an exogenous donor of $H_2S$, while DL-propargylglycine (PAG) is an irreversible inhibitor of CSE, which is responsible for $H_2S$ generation in the cardiovascular system.[13] In recent years, evidence shows that $H_2S$ has an increasingly salient contribution in the physiological and pathological processes of the heart.[14] Recent evidence suggests that $H_2S$ has protective properties against inflammation and cell death in the diabetic heart, although it does not reduce high blood glucose level.[15] Exogenous $H_2S$ is also reported to protect H9c2 cardiomyocytes from high glucose-induced damage by suppressing NLRP3 inflammasome activation.[16] However, in type 1 diabetic rats, whether $H_2S$ protects the myocardium against injury by inhibiting NLRP3 inflammasome activation remains unclear. Hence, in this work, we explored the therapeutic effect of $H_2S$ on cardiac inflammation in diabetic rats and elucidated its mechanism, i.e. whether it is related to inhibition of TXNIP-mediated NLRP3 inflammasome activation.

## Materials and methods

### Animals

Twenty-eight healthy male Sprague-Dawley rats (weighing 180–220 g) aged 7–8 weeks were acquired from the Experimental Animal Center of Bengbu Medical College, Bengbu, China. All the animals were kept at a room temperature-controlled environment at $23 \pm 1^{\circ}C$ under a 12 h light–12 h dark cycle and were offered a standard rodent diet and water *ad libitum*. All experimental procedures were approved by the Animal Experimentation Ethics Committee of Bengbu Medical College.

### Chemicals and reagents

NaHS, PAG, and streptozotocin (STZ) were provided by Sigma-Aldrich, USA. Creatine kinase (CK), glutathione peroxidase (GSH-Px), catalase, malondialdehyde (MDA), total antioxidant capacity (T-AOC), lactate dehydrogenase (LDH), superoxide dismutase (SOD), and lipid peroxidation (LPO) assay kits were acquired from Jiancheng Bioengineering Institute, Nanjing, China. ELISA kits for detection of IL-6, IL-18, tumor necrosis factor (TNF)-α, and IL-1β were provided by Neobioscience, Shenzhen, China. Primary antibodies against Thioredoxin and TXNIP were provided by Abcam, UK. Primary antibody against ASC was provided by Santa Cruz Biotechnology, USA. Primary antibodies against NLPR3, caspase-1 (p20), and β-actin and secondary antibodies were provided by Boster Biotechnology, Wuhan, China.

### Experimental design

Animals were arranged randomly into four groups of seven rats each: control (CON), STZ, STZ + NaHS (SH), and STZ + PAG (SP). A rat model of type 1 diabetes was established in accordance with our previously reported method.[13] Briefly, the overnight-fasted animals in the three STZ groups were injected intraperitoneally (i.p.) with 55 mg/kg STZ. Then, 72 h after STZ injection, the overnight-fasted animals with hyperglycemia (blood glucose over 16.7 mM) were considered diabetic. From the fifth week after successful establishment of the diabetes model, the rats assigned to the SH and SP groups were administrated NaHS (56 μmol/kg/day) and PAG (40 mg/kg/day), i.p., respectively, for 28 days. Animals assigned to the other two groups were administrated with the same volume of 0.9% NaCl solution i.p. for the same duration. The level of fasting blood glucose (FBG) was monitored weekly during the experimental period.

### Cardiac function measurement

After different treatments, rats were weighed and anesthetized with an injection i.p. of 350 mg/kg chloral hydrate. Cardiac function-related parameters, including left ventricular (LV) end-diastolic pressure (LVEDP), LV systolic pressure (LVSP), as well as maximal rise/fall rate of LV pressure ($\pm dp/dt_{max}$), were measured using the method we previously described.[13]

### Determination of FBG and heart-weight/body-weight

After cardiac function measurement, the blood sample from each rat was gathered and the FBG level was measured. After rats were sacrificed, their hearts were removed and weighed. Then the heart-weight (HW)/body-weight (BW) ratio was calculated.

### Determination of activities of cardiac enzyme

The centrifuge tubes containing blood were centrifuged and the sera were subsequently harvested to examine CK and LDH activities using protocols supplied by the manufacturer.

### Determination of levels of serum inflammatory cytokines

Serum inflammatory cytokines including IL-6, TNF-α, IL-1β, and IL-18 were detected using ELISA kits in accordance with the protocols of corresponding assay kits.

### Histopathological and ultrastructural evaluation

For histopathological analysis, fresh cardiac tissues were harvested, soaked in 4% paraformaldehyde solution, and subsequently fixed in paraffin wax. Heart slices (5 μm) were dyed with hematoxylin and eosin (H&E) staining kit (Beyotime Biotechnology, China) and Masson's trichrome reagents, respectively. Sections were examined with a NanoZoomer 2.0 RS digital pathology slide scanner (magnification, ×400).

For the ultrastructural study, fresh heart tissue was cut into the size of 1 mm × 1 mm × 1 mm, and the ultrathin slices were made and stained in accordance with the reported method.[13] These ultrathin slices were analyzed under a JEM-1230 transmission electron microscope.

### Estimation of biochemical indicators

Heart tissues (100 mg) were homogenized in a nine-fold volume of freezing 0.9% NaCl solution. After centrifugation, the supernatant fluids were obtained. After determining the protein concentrations, the myocardial T-AOC, catalase, SOD, GSH-Px, MDA, and LPO levels were examined using the corresponding assay kits.

### Immunohistochemical analysis

Five micrometer-thickness myocardial slices embedded in paraffin wax were dewaxed and then rehydrated. After heating for antigen retrieval, the slices were covered with 3% hydrogen peroxide to prevent the activity of myocardial peroxidase. The sections were treated with 3% bovine serum albumin and subsequently treated with anti-Thioredoxin and anti-TXNIP rabbit polyclonal antibodies, respectively. All the myocardial slices were then incubated with secondary immunoglobulin G (IgG) antibody. After washing with PBS, sections were treated using diaminobenzidine and then dyed with hematoxylin, dehydrated, and mounted. Immunohistochemical results were observed and imaged by the digital pathology slide scanner (magnification, ×400). Four views of each slice were chosen randomly and calculated via Image Pro-Plus 6.0 software.

### Western blot analysis

Cardiac tissue lysate was extracted using a lysis buffer with phenylmethanesulfonyl fluoride, and the myocardial protein content was quantified by a bicinchoninic acid kit. A total of 40 μg of proteins were isolated using SDS-PAGE and subsequently electroblotted onto polyvinylidene difluoride membranes. After immersing with 5% nonfat milk powder in Tris-buffered saline Tween-20 for 2 h, all the membranes were probed with primary antibodies against NLRP3, β-actin, ASC, and caspase-1 (p20) at 4°C, overnight. Subsequently, membranes were immersed in proper horseradish peroxidase-linked secondary IgG antibodies. Finally, the immunoreactive band was visualized after incubation with an enhanced chemiluminescence solution and then imaged using a ChemiDoc XRS system. The relative band density was normalized to that of the β-actin loading CON and analyzed using Quantity One software.

### Statistical analysis

Statistical analyses were done with SPSS 17.0 software. Experimental results were shown as means ± SD. Data were analyzed using one-way analysis of variance followed by the Newman-Keuls test. Statistical significance was set at *P*-value less than 0.05.

## Results

### Effect of $H_2S$ on FBG and HW/BW

As shown in Figure 1, FBG and HW/BW were increased remarkably, whereas BW and HW were decreased in the STZ group compared with the CON group, which indicated that diabetes causes cardiac hypertrophy. Compared with the STZ group, except for FBG, NaHS treatment reduced the ratio of HW/BW, and increased BW and HW in the SH group. On the other hand, there were no statistically significant differences in FBG, HW, and HW/BW, whereas BW was decreased in the SP group in contrast to the STZ group. The findings showed that $H_2S$ ameliorated cardiac hypertrophy in diabetic rats.

### Effect of $H_2S$ on cardiac function

Significant impairment of cardiac function was observed in the STZ group, including a rise in LVEDP and a decline in LVSP and $\pm dp/dt_{max}$. NaHS treatment enhanced cardiac systolic and diastolic function remarkably in contrast to the STZ group. On the other hand, PAG treatment aggravated cardiac systolic and diastolic dysfunction in contrast to the STZ group (Figure 2).

### Effects of $H_2S$ on cardiac enzymes and inflammatory cytokines in serum

As shown in Figure 3, STZ injection to rats resulted in cardiac damage and inflammatory reaction; the levels of serum cardiac enzymes (CK and LDH) and pro-inflammatory factors (IL-6, TNF-α, IL-18, and IL-1β) were raised remarkably in the STZ group in contrast to the CON group. On treatment with exogenous $H_2S$, the levels of cardiac enzymes and pro-inflammatory factors in serum were significantly reduced in the SH group in contrast to the STZ group. On the other hand, on treatment with PAG to inhibit the production of endogenous $H_2S$, the same indicators in serum were further raised in the SP group in

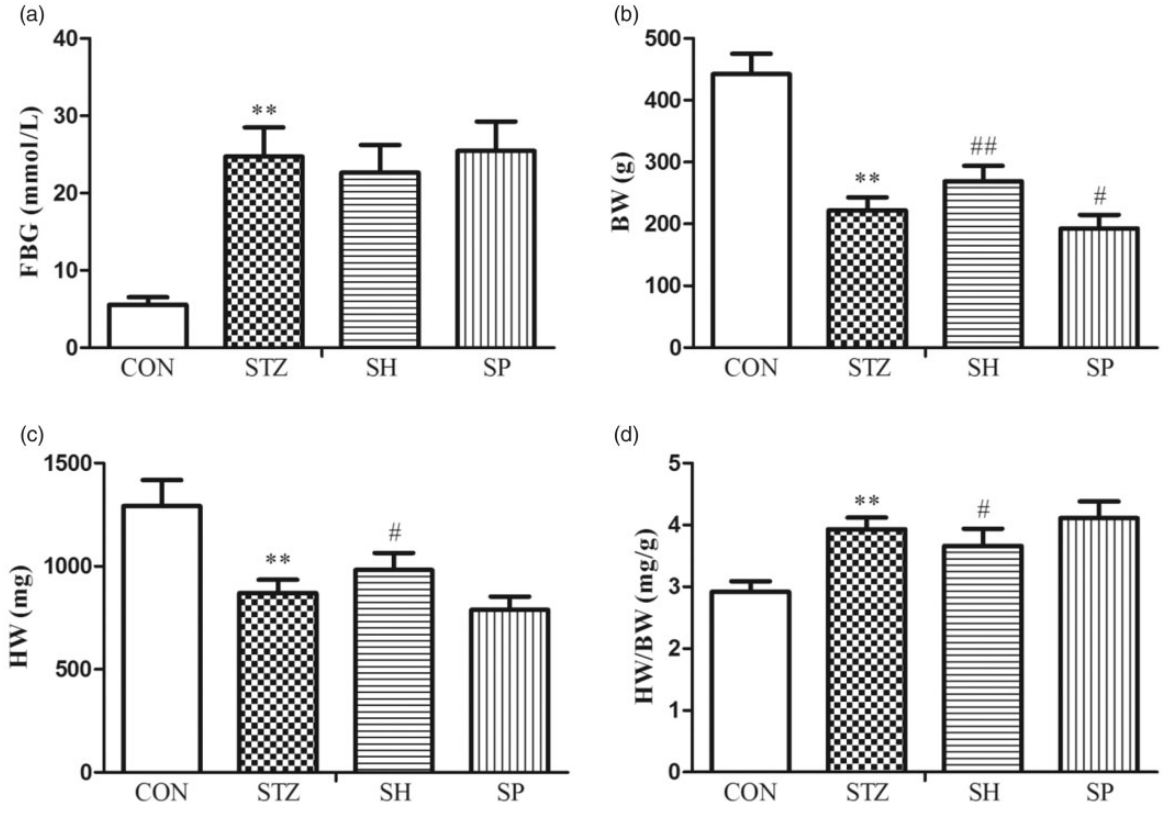


**Figure 1.** Effects of $H_2S$ on FBG and HW/BW in rats. (a) FBG. (b) BW. (c) HW. (d) HW/BW. Values, mean ± SD; $n = 7$; $^{**}P < 0.01$ vs. CON group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. STZ group; CON: control group; STZ: streptozotocin group; SH: STZ + sodium hydrosulfide group; SP: STZ + DL-propargylglycine group.

contrast to the STZ group, suggesting that $H_2S$ could ameliorate STZ-induced cardiac damage and inflammatory reaction.

### Effect of $H_2S$ on histopathological and ultrastructural alterations

H&E staining revealed that the myofibers were arranged neatly and orderly, with clear cell nuclei and minimal inflammatory cell infiltration in the normal heart tissue. The myofibers were disordered with inflammatory cell infiltration in cardiac tissue in the STZ group. Compared with the STZ group, on treatment with NaHS, myocardial injury was evidently alleviated, and most of the myofibers were arranged in neat rows, and infiltration of inflammatory cells in the SH group was further reduced; after treatment with PAG, the myocardial injury deteriorated further in the SP group (Figure 4(a)).

Masson's trichrome staining showed normal cardiac myocytes with less collagen deposition in the CON group. A lot of blue-stained collagen fibers were deposited in the diabetic hearts in the STZ group. On treatment with exogenous $H_2S$, the deposition of collagen fibers evidently declined in the SH group, whereas the deposition of collagen fibers was further increased in the SP group in contrast to the STZ group (Figure 4(b)).

Ultrastructural examination of the normal hearts showed normal architecture of cardiac myocytes. The integrity of the cardiomyocytes in diabetic sections was destroyed, the myofibers were partially broken and even dissolved, and the mitochondria were swollen. In the SH group, the ultrastructural injury was evidently ameliorated, whereas the severity of cardiomyocyte injury was further aggravated in the SP group (Figure 4(c)).

### Effect of $H_2S$ on redox status in DCM

STZ injection to rats resulted in hyperglycemia-triggered oxidative stress and disturbed the balance of redox state; the results showed that the level of T-AOC and the activities of catalase, GSH-Px, and SOD were decreased, whereas the levels of MDA and LPO were raised in myocardial tissues from diabetic rats in contrast to the CON group. Treatment with NaHS downregulated the oxidative status of myocardial tissue in the SH group in contrast to the diabetic rats. On the other hand, after treatment with PAG, the above-mentioned indicators further deteriorated in the SP group compared with the diabetic rats (Figure 5).

Immunohistochemical results exhibited that compared with the CON group, the expression level of Thioredoxin protein was reduced, whereas the expression level of TXNIP protein was raised in myocardial tissue from the STZ group. On treatment with NaHS, the expression level of Thioredoxin protein was elevated, whereas the expression level of TXNIP protein was reduced remarkably in the SH group in contrast to the diabetic rats. However, treatment with PAG significantly promoted TXNIP expression

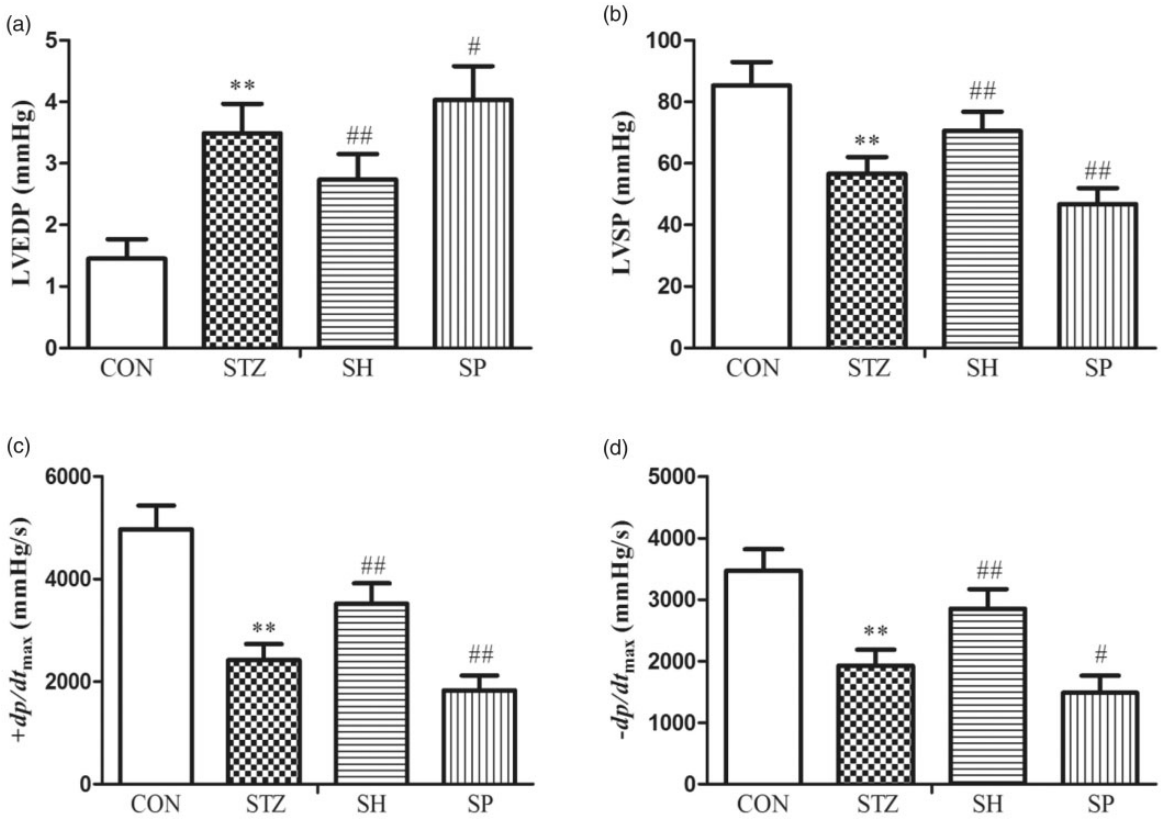


**Figure 2.** Effects of $H_2S$ on cardiac functional parameters in rats. (a) LVEDP. (b) LVSP. (c) $+dp/dt_{max}$. (d) $-dp/dt_{max}$. Values, mean ± SD; $n = 7$; $^{**}P < 0.01$ vs. CON group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. STZ group; CON: control group; STZ: streptozotocin group; SH: STZ+sodium hydrosulfide group; SP: STZ+DL-propargylglycine group.

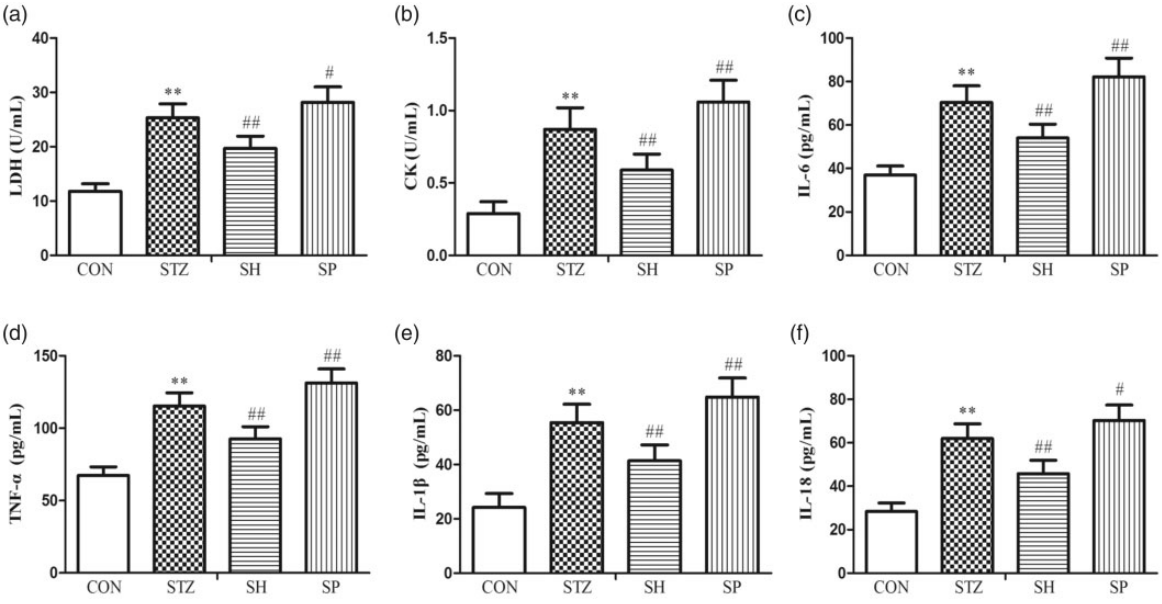


**Figure 3.** Effects of $H_2S$ on cardiac enzymes and inflammatory cytokines in serum. (a) LDH. (b) CK. (c) IL-6. (d) TNF-α. (e) IL-1β. (f) IL-18. Values, mean ± SD; $n = 7$; $^{**}P < 0.01$ vs. CON group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. STZ group; CON: control group; STZ: streptozotocin group; SH: STZ+sodium hydrosulfide group; SP: STZ+DL-propargylglycine group.

further and reduced Thioredoxin expression in the SP group in contrast to diabetic rats (Figure 6). These findings showed that $H_2S$ improved the balance of the redox status in diabetic cardiomyocytes.

### Effect of $H_2S$ on the NLRP3 inflammasome in DCM

Protein expression levels of NLRP3, activated caspase-1, and ASC were measured to elucidate the activation status of NLRP3 inflammasome. Immunoblotting results

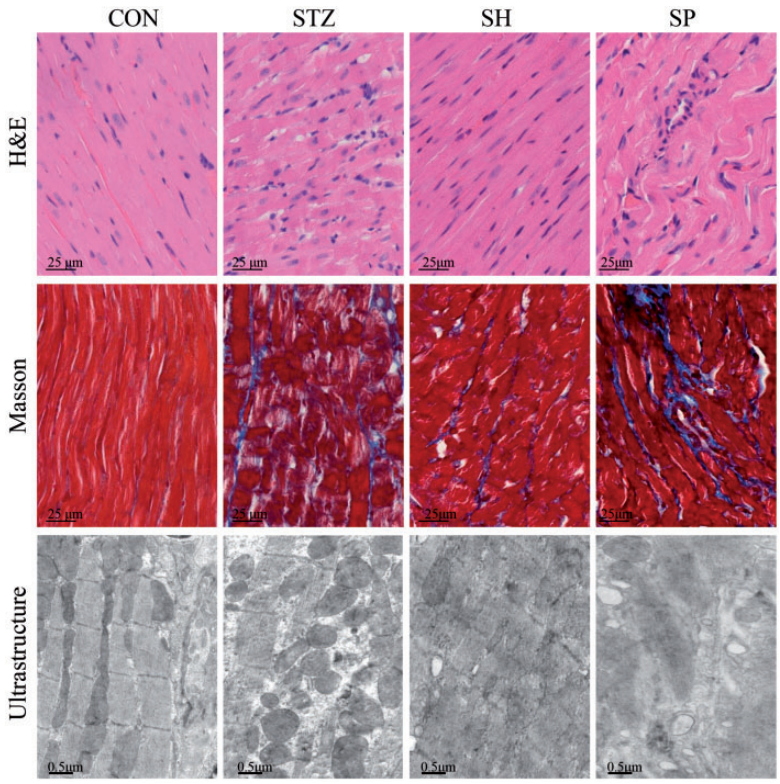


**Figure 4.** Effects of $H_2S$ on H&E staining (×400), Masson's trichrome staining (×400), and ultrastructural alterations (×8000) in myocardial tissues. CON: control group; STZ: streptozotocin group; SH: STZ+sodium hydrosulfide group; SP: STZ+DL-propargylglycine group. (A color version of this figure is available in the online journal.)

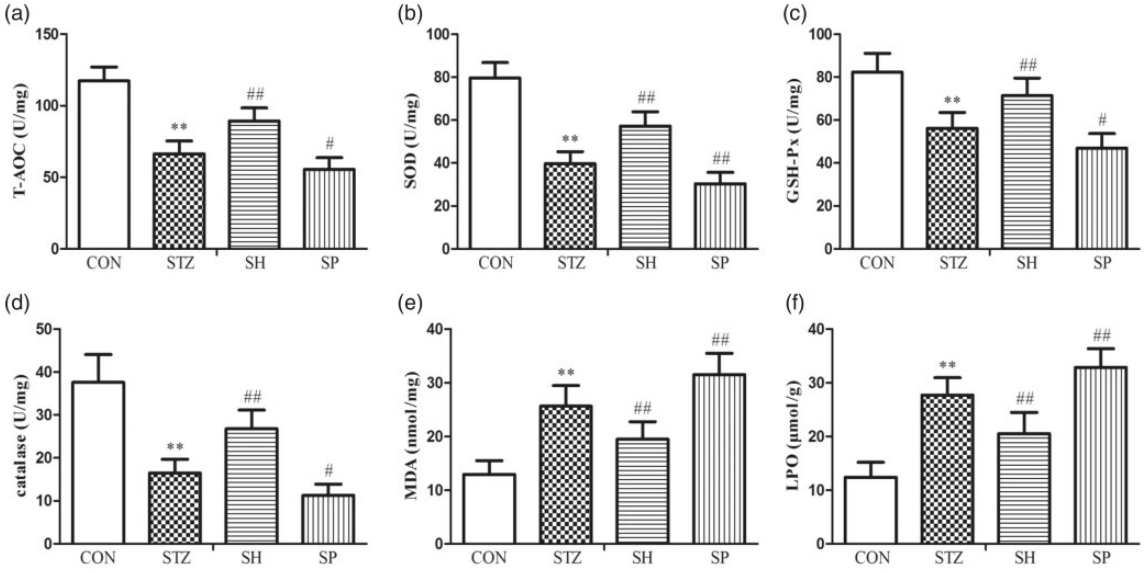


**Figure 5.** Effects of $H_2S$ on redox status in myocardial tissue in rats. (a) T-AOC. (b) SOD. (c) GSH-Px. (d) catalase. (e) MDA. (f) LPO. Values, mean ± SD; $n = 7$; $^{**}P < 0.01$ vs. CON group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. STZ group; CON: control group; STZ: streptozotocin group; SH: STZ + sodium hydrosulfide group; SP: STZ + DL-propargylglycine group.

exhibited that there was significant activation of NLRP3 inflammasome in the STZ group in contrast to the CON group. Exogenous $H_2S$ downregulated the expression levels of NLRP3, caspase-1, and ASC proteins, whereas treatment with PAG significantly upregulated the expression levels of NLRP3 inflammasome-associated proteins compared with diabetic rats (Figure 7). The results exhibited that $H_2S$ downregulated the activation status of NLRP3 inflammasome in diabetic cardiomyocytes.

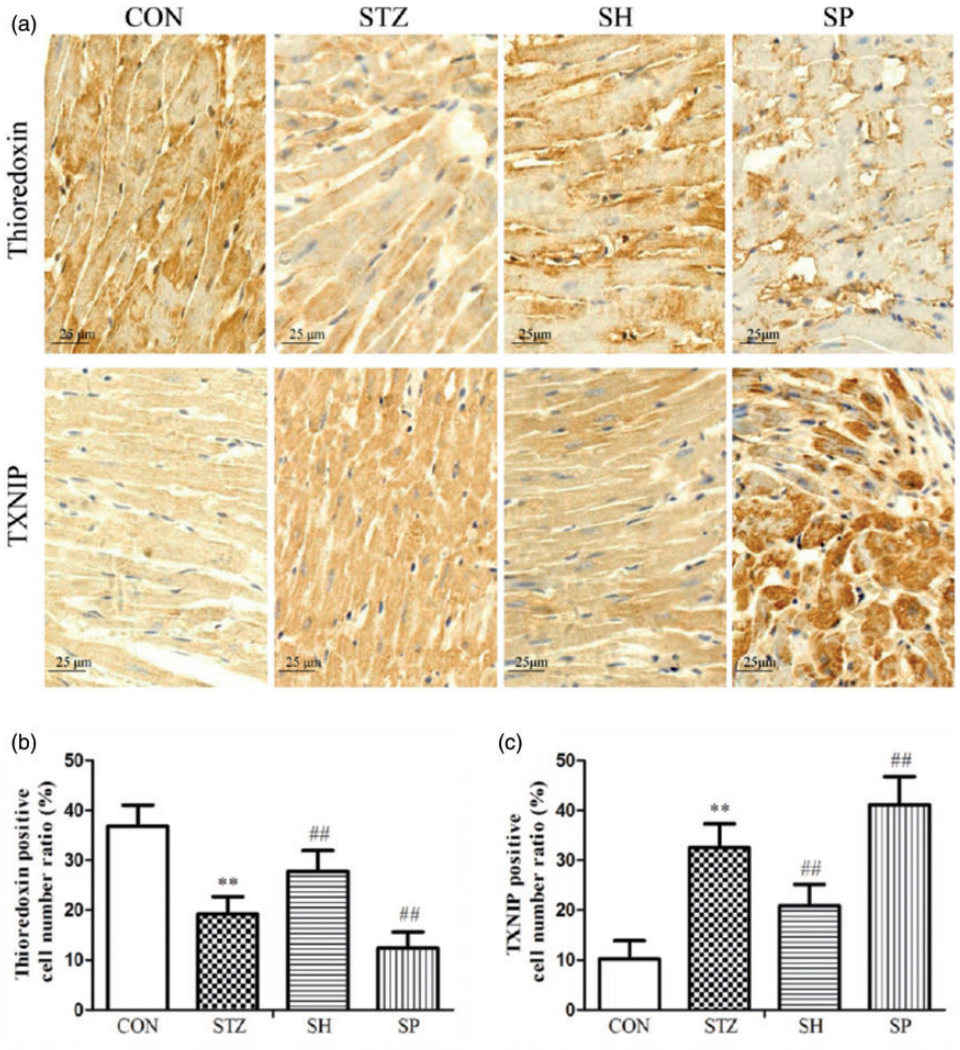


**Figure 6.** Effects of $H_2S$ on the protein expressions of Thioredoxin and TXNIP in myocardial tissues. (a) Immunohistochemical studies of Thioredoxin and TXNIP were accessed in rat myocardial tissues of each group (×400). (b) The ratio of Thioredoxin-positive cells in rat myocardial tissues of each group. (c) The ratio of TXNIP-positive cells in rat myocardial tissues of each group. Values, mean ± SD; $n = 7$; $^{**}P < 0.01$ vs. CON group; $^{\#\#}P < 0.01$ vs. STZ group; CON: control group; STZ: streptozotocin group; SH: STZ+sodium hydrosulfide group; SP: STZ+DL-propargylglycine group. (A color version of this figure is available in the online journal.)

## Discussion

DCM is a heart disease specifically associated with diabetes mellitus and is mainly characterized by cardiac systolic and diastolic dysfunction.[17] Cardiac hypertrophy and myocardial histological and ultrastructural damage are observed in the diabetic heart.[18] Among these, myocardial fibrosis is also a key pathological hallmark of DCM.[19] In addition, CK and LDH are usually considered as the biochemical markers of myocardial injury.[20] Briefly, these indicators are commonly used to assess the degree of diabetic myocardial damage. In the current work, we utilized STZ to establish a type 1 diabetic rat model. Our experimental results exhibited that compared with normal rats, cardiac function deteriorated, whereas the levels of FBG, CK, and LDH increased in diabetic rats. As expected, cardiac hypertrophy, myocardial histological injury, and fibrosis were observed in the diabetic rats, indicating that the animal model of DCM was established successfully.

Hyperglycemia, as the leading characteristic of diabetes mellitus, causes myocardial injury through induction of oxidative stress, inflammation, and even cell death.[3] Increased metabolism of high glucose through mitochondrial glucose oxidation contributes to the over-generation of reactive oxygen species (ROS) in diabetes. ROS react with polyunsaturated fatty acids to generate lipid peroxides, and, thus, the content of LPO and MDA reflects the state of oxidative stress injury.[21] T-AOC represents the total antioxidant capacity, while some antioxidant enzymes, including GSH-Px, catalase, and SOD, are usually regarded as the indicators of reductive capability in mammals. Furthermore, Thioredoxin is an important antioxidant protein *in vivo*.[22] TXNIP has been reported to promote oxidative stress by combining with and inhibiting Thioredoxin.[23] In this rat model of diabetes, the results showed that myocardial T-AOC, the activities of antioxidant enzymes, and Thioredoxin expression were decreased, whereas the levels of MDA, LPO, and TXNIP expression were increased in the

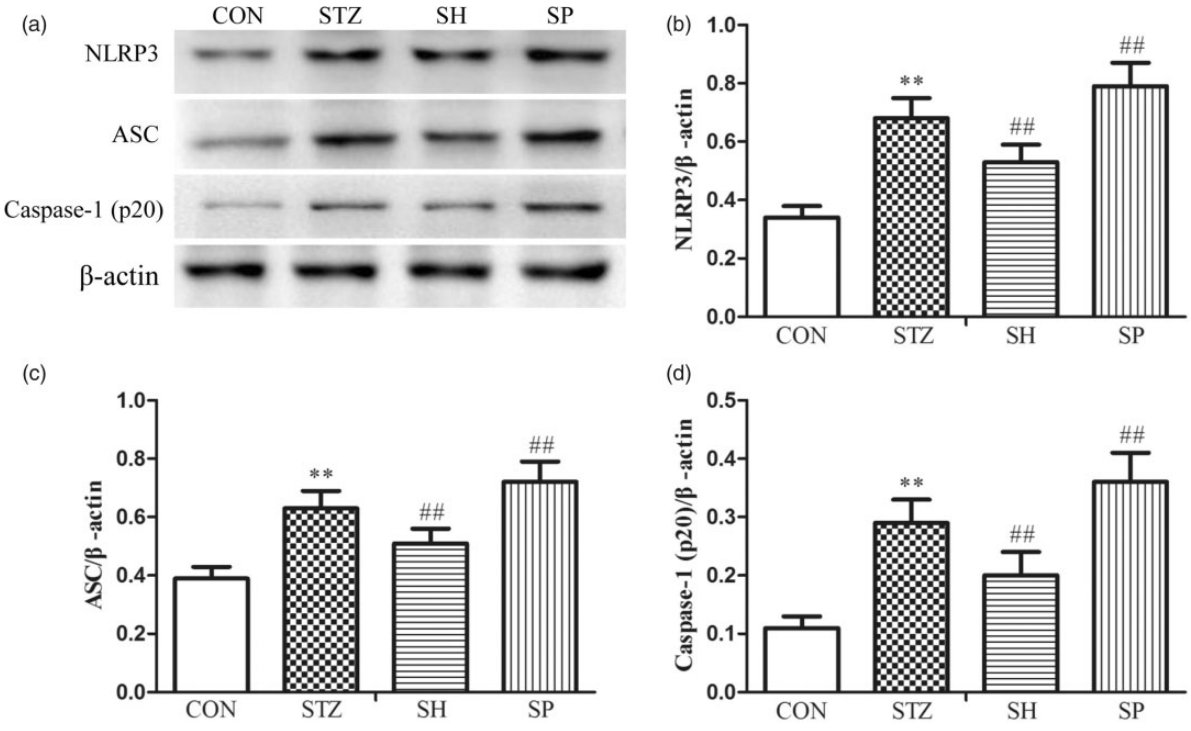


**Figure 7.** Effects of $H_2S$ on NLRP3 inflammasome expression in rat myocardial tissues of each group. (a) Representative western blotting showing NLRP3, ASC, and caspase-1 (p20) alterations. (b–d) Quantitative analyses of NLRP3, ASC, and caspase-1 (p20) proteins. β-actin was used for normalization of protein expression levels. Values, mean ± SD; $n = 7$; $^{**}P < 0.01$ vs. CON group; $^{\#\#}P < 0.01$ vs. STZ group; CON: control group; STZ: streptozotocin group; SH: STZ+sodium hydrosulfide group; SP: STZ+DL-propargylglycine group.

diabetic myocardia compared with normal myocardia, which indicated that hyperglycemia caused TXNIP-mediated oxidative stress injury in diabetic cardiomyocytes.

$H_2S$ is a novel endogenous gas messenger that plays a crucial role in the various pathophysiological processes in mammalian organisms, such as in the cardiovascular system, nervous system, and urinary system.[24–26] $H_2S$ has been reported to exert anti-oxidative, anti-inflammatory, and anti-apoptotic activities in diabetic myocardium.[27] The concentration of $H_2S$ is essential to the exertion of its protective effects *in vivo*.[13] Evidence suggests that NaHS treatment inhibits TXNIP expression, whereas PAG treatment upregulates TXNIP expression in endothelial cells.[28] This study found that exogenous $H_2S$ significantly alleviated diabetes-induced cardiac hypertrophy and oxidative stress, enhanced cardiac function, ameliorated histomorphological damage, and downregulated cardiac injury enzyme (CK and LDH) levels in the SH group. However, once the generation of endogenous $H_2S$ was suppressed with PAG, oxidative stress, cardiac dysfunction and injury, and histomorphological damage were further aggravated in the SP group. The current results exhibited that by improving the balance of redox status in diabetic myocardia, $H_2S$ had protective effects against diabetes-induced damage.

Recent evidence reveals that oxidative stress stimulates chronic inflammatory reaction in the diabetic myocardium.[29] This inflammatory reaction is one of the most important mechanisms leading to diabetic myocardial damage.[30] IL-6 and TNF-α are important proinflammatory mediators *in vivo*.[31] This work showed that infiltration by inflammatory cells and levels of IL-6 and TNF-α were increased in the STZ group, which indicated that inflammation existed in the diabetic myocardium. After treatment with NaHS, the inflammatory reaction was reduced remarkably in the SH group. However, in the SP group, the inflammatory reaction was further aggravated in contrast to the STZ group, which demonstrated that $H_2S$ exerted an anti-inflammatory role in diabetic cardiomyocytes.

Inflammasomes are involved in the inflammatory process and even cause myocardial injury.[32] Among many known inflammasome complexes, the NLRP3 inflammasome complex is well studied and comprises of NLRP3, procaspase-1, and ASC.[33] Upon activation of NLRP3, procaspase-1 is cleaved to release active caspase-1, which cleaves proinflammatory mediator precursors, pro-IL-1β and pro-IL-18, into their active forms, further exacerbating the inflammatory process.[34] Furthermore, the involvement of TXNIP is important for the regulation of NLRP3 inflammasome.[35] Under physiological conditions, TXNIP combines with Thioredoxin and does not interact with NLRP3 protein. Once excessive ROS stimulation occurs, TXNIP is separated from Thioredoxin and then combines with NLRP3 protein, regulating the activity of the NLRP3 inflammasome positively. The NLRP3 inflammasome is a critical contributor to the innate immune response and has become a key regulator of inflammatory reaction in diabetes mellitus.[36] Inhibition of NLRP3 inflammasome activation significantly alleviated myocardial injury in a rat model of DCM.[37] This study found that compared with normal rats, there was decreased expression level of

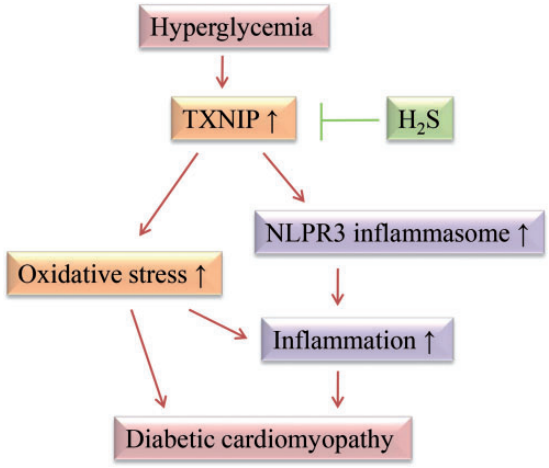


**Figure 8.** Possible mechanism of $H_2S$ against TXNIP-mediated NLRP3 inflammasome activation in diabetic cardiomyopathy. (A color version of this figure is available in the online journal.)

Thioredoxin and increased expression levels of TXNIP and NLRP3 inflammasome-related proteins in diabetic hearts. After treating the diabetic rats with exogenous $H_2S$, the protein expression level of Thioredoxin was raised, whereas the expression of TXNIP and NLRP3 inflammasome complex-associated proteins was reduced in myocardial tissue in the SH group. On the other hand, when the generation of endogenous $H_2S$ was suppressed by PAG, the above-mentioned indicators deteriorated in the SP group, further demonstrating that the concentration of $H_2S$ was important for the exertion of its protective effects. The current work indicated that $H_2S$ exerted an anti-inflammatory role in diabetic myocardia by downregulation of TXNIP-mediated NLRP3 inflammasome activation.

In conclusion, $H_2S$ alleviated hyperglycemia-mediated myocardial inflammation in type 1 diabetes. As illustrated in Figure 8, the mechanism may involve the inhibition of TXNIP-mediated NLRP3 inflammasome activation. This evidence suggests that strategies aimed at inhibiting TXNIP-mediated NLRP3 inflammasome by use of exogenous $H_2S$ might serve as efficient targeted therapy in diabetic cardiomyocytes.

**Authors' contributions:** QJ, SM, XL, SM, and RY designed the experiments, carried out the studies, participated in collecting data, and drafted the manuscript. QJ, XL, and SM analyzed the statistical results. SM and RY revised the manuscript.

## DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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