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*Original Article*

# Effect and mechanism of Huangqi gegen decoction (HGD) on diabetic cardiomyopathy of diabetic rats

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**Abstract:** Huangqi gegen decoction (HGD) has been reported to exhibit protective effects against various cardiovascular disease models. However, the role little is currently known regarding the role and mechanism of HGD on diabetic cardiomyopathy. In the present study, healthy male Wistar rats were injected with STZ to induce diabetic rats. We found that a significant increase in the body weight (BW) and lower heart weight (HW)/BW in DM HGD-treated group when compared to DM group, and the LVWI also significantly decrease after treated with HGD. And the levels of CK-MB, BNP, TNF-α and IL-6 as well as the mRNA and protein expression levels of Collagen I and MMP2 were lower in the DM HGD-treated group, compared with the rats in the DM group ($P<0.05$). In addition, The DM group showed higher the phosphorylation of Akt and NF-κb compared to the control group, but significantly decrease after treated with HGD. These results suggest that HGD play important role in ameliorating diabetic cardiomyopathy by affecting fibrosis and inflammation, and that the activation of Akt/NF-κb may be a key mechanism in the protection conferred by HGD.

**Keywords:** Huangqi gegen decoction (HGD), diabetic cardiomyopathy, cardiac fibrosis

## Introduction

Dabetes mellitus (DM) is becoming one of the most severe public health problem in Asia, especially in China [1]. Diabetic cardiomyopathy (DCM) is a unique cardiovascular Disease, which is characterized with an increase in cardiac mass in response to applied stimulus. Diabetic patients are at an increased risk of cardiovascular diseases and these are the major cause of death in them, is the leading cause of mortality among patients with diabetes [2, 3]. However, the development of DCM has been poorly understood and the mechanisms underlying have not been completely elucidated. There is enough evidence today to suggest that many factors are important for the development of Diabetic cardiomyopathy (DCM), including hyperglycemia and the resulting oxidative stress, inflammation, cardiac fibrosis and myocardial apoptosis, etc [4]. Hence, it is important to improve hyperglycemia and reduce cardiac fibrosis and inflammation, which might retard the progression of diabetic cardiomyopathy.

Natural products are considered particularly attractive antiobesity drug candidates because of their higher efficacies and fewer side effects [5]. Chinese medicine comprised of all natural products is widely used to treat diabetes and its complications in the local clinics of China [6]. Huangqi gegen decoction (HGD), which has been reported that is the most common herbal medicine in treating of the common cold, flu, and fever, etc [7]. Huangqi Gegen decoction has been reported to have potentially beneficial effects in the treatment of diabetes in animal trials, as well as in some clinical observations [8, 9]. For example, Gegen decoction significantly reduced FBG and HbA1c in STZ-induced diabetic rats [10], but the underlying mechanism of it was not unclear.

In the present study, we investigate whether Huangqi gegen decoction (HGD) prevent diabet-

ic cardiomyopathy and the underlying mechanism in the Wistar mice hearts.

## Materials and methods

### *Animals and experimental design*

Eight-week-old male Wistar rats were purchased from Laboratory Animal Science of Chinese Academy of Medical Sciences (Beijing, China). All mice were housed in same controlled conditions under temperature of 25±1°C and humidity of 55%±5% with 12/12-hour light-dark cycles. The animals were randomly divided into four groups: diabetic (DM) group, DM+100 mg/kg HGD group, DM+200 mg/kg group, control group, with 8 rats each group. DM was induced in 40 rats via intraperitoneal injection of 70 mg/kg streptozotocin (STZ; dissolved in 0.1 M citrate buffer; Sigma-Aldrich, St. Louis, MO, USA). After three days of STZ injections, the blood glucose levels were measured using a glucometer (LifeScan, USA). Rats with fasting blood glucose >11.1 mmol/L in two consecutive analyses were considered the diabetic rat model ones. Rats in the DM+HGD group were intraperitoneally injected with 50 or 100 mg/kg/day, while DM group and control injected with vehicle. After 6 weeks of diabetes, the mice were sacrificed, heart was removed and plasma was collected for further studies.

### *Measurements of HW/BW and left ventricular mass index (LVMI)*

Heart weight (HW), left ventricle mass (LVM), and body weight (BW) were determined, and calculated HW/BW and left ventricle mass/BW were calculated.

### *Measurement of BNP and CK-MB in the plasma*

Serum BNP and CK-MB levels in the rats were measured by enzyme-linked immunosorbent assay (ELISA) and enzyme rate assay according to the manufacturer's instructions, and decked by electrochemical analyzer.

### *Measurement of TNF-α and IL-1b in the plasma*

Plasma TNF-α and *IL-1b* levels were determined using a sandwich ELISA method with a commercially available kit (BD Biosciences, NJ, USA). Samples were acidified with 5 M HCl, incubated for 30 minutes at 37°C, and then neutralized with 1.4 M NaOH. Appropriate controls and standards were used, as specified by the manufacturer's instructions.

### *Real-time PCR*

The total RNA was extracted from cardiac tissue with TRIzol reagent according to the manufacturer's instructions (Invitrogen). 2 μg total RNA was used to synthesize the cDNA using reverse transcription kit (Promega, WI, USA) according to the manufacturer's instructions. qPCR was carried out with a 7500 Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA) using SYBR-Green I (Applied Biosystems) as a fluorescent dye according to the manufacturer's instructions. Primers for Type I collagen, MMP-2 and β-actin were synthesized by Sangon Biotech Shanghai Co., Ltd. Collagen I: Forward Primer 5'-AGGGACCCTTAGGCCATTGTGTA-3', Reverse Primer 5'-GACATGTTCAGCTTTGTGGACCTC-3'; MMP-2: Forward Primer 5'-GG ACAAGTGGTCCGCGTAAA-3', Reverse Primer 5'-CCGACCGTTGAACAGGAG G-3'. β-actin: Forward Primer 5'-GGCTGTATTCCCCTCCATCG-3', Reverse Primer 5'-CCAGTTG GTAACAATGCCATGT-3'. Samples were tested for three times, and the average values were used for quantification. The relative expression level of each target gene was determined by $2^{-\Delta\Delta CT}$ method with GAPDH as the internal reference.

### *Western blot analysis*

Myocardial tissue was cut into fragments, and then homogenized with RIPA buffer (Beijing Solarbio Science and Technology Co., Ltd, China) for protein extraction. Protein concentration was determined using BCA Protein Assay Kit according to the manufacturer's protocol. Denaturized proteins from samples were loaded to 10% SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membrane. Membranes were incubated overnight at 4°C with the primary antibodies for overnight, followed by peroxidase-conjugated IgG polyclonal antibody for 2 h at room temperature. Blots were developed by ECL kit (Pierce Biosciences, USA). The antibody of phospho-AKT, AKT, phospho-NF-κb and NF-κb purchased from Cell Signaling Technology, Inc (Danvers, MA, USA), β-actin, Type I collagen and MMP-2 antibody purchased from Santa Cruz Biotechnology, Inc (Delaware Avenue, CA, USA).

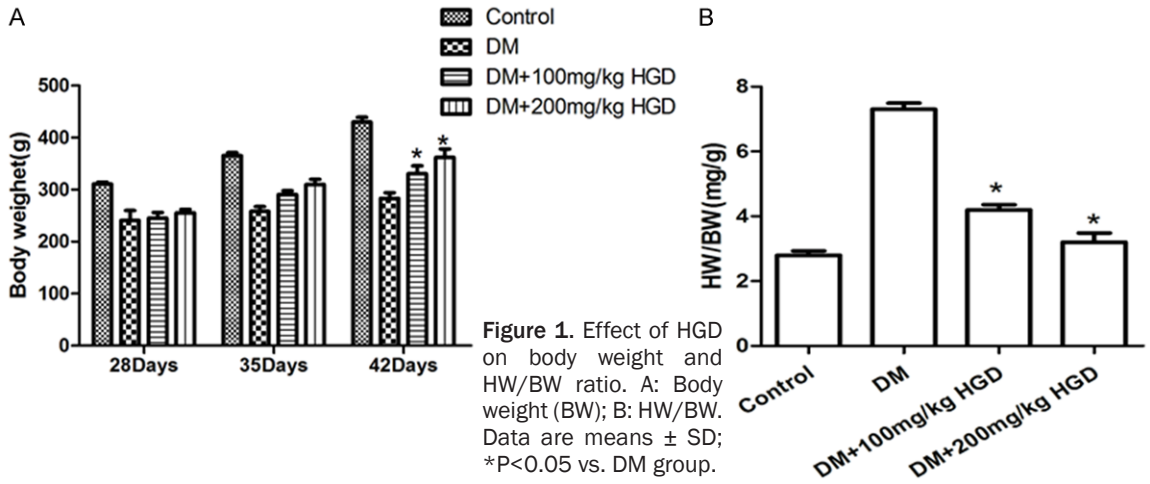


**Figure 1.** Effect of HGD on body weight and HW/BW ratio. A: Body weight (BW); B: HW/BW. Data are means ± SD; *P<0.05 vs. DM group.

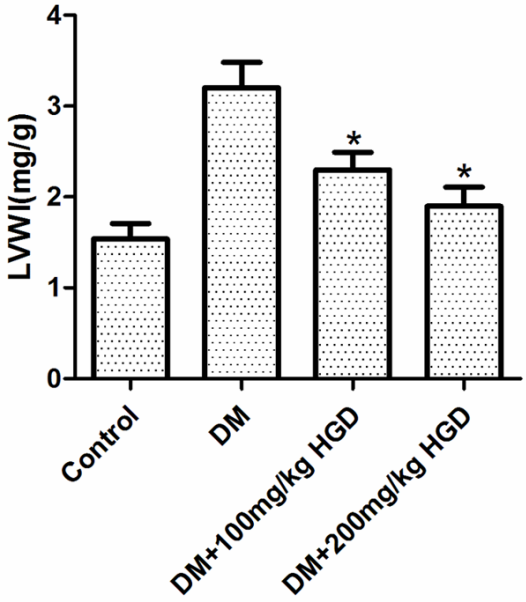


**Figure 2.** Effect of HGD on *LVWI*. Data are means ± SD; *P<0.05 vs. DM group.

*Statistical analysis*

All data are expressed as mean ± SD and analyzed by one-way analysis of variance (ANOVA). All analyzes were performed using the statistical software SPSS 18.0 (Chicago, IL, USA). A probability of P<0.05 was considered to be a significant difference.

## Results

*HGD increase the BW in diabetic rats*

As showed in **Figure 1A**, compared with the control group, a significant decrease in the BW was observed in the DM group (P<0.05), DM+100 mg/kg HGD group (P<0.05), and DM+200 mg/kg HGD group (P<0.05). When compared with the DM group, a significant increase in the BW was observed DM+100 mg/kg HGD group (P<0.05) and DM+200 mg/kg HGD group (P<0.05). These results indicate that Huangqi gegen decoction (HGD) may have a role in BW. The results in **Figure 1B** showed that HW/BW in DM+100 mg/kg HGD group and DM+200 mg/kg HGD group significantly decrease when compared to DM group, but increased in control group.

*HGD decreases the LVWI in diabetic rats*

Compared with the control group, a significant increase in the LVWI was observed in the DM group (P<0.05), DM+100 mg/kg HGD group (P<0.05), and DM+200 mg/kg HGD group (P<0.05). After treated with 100 mg/kg HGD or 200 mg/kg HGD, the LVWI decrease significantly compared to the DM group. These results indicate that Huangqi gegen decoction (HGD) could decrease the LVWI level in rats (**Figure 2**).

*HGD decreases the levels of CK-MB and BNP*

Compared to the control group, there was a significant increase in CK-MB in the DM groups (P<0.05). After treated with 100 mg/kg HGD or 200 mg/kg HGD, the CK-MB levels decreased significantly compared to the DM group. Compared with the DM group, 100 mg/kg HGD or 200 mg/kg HGD treated group gained significantly lower BNP (P<0.05) (**Figure 3**).

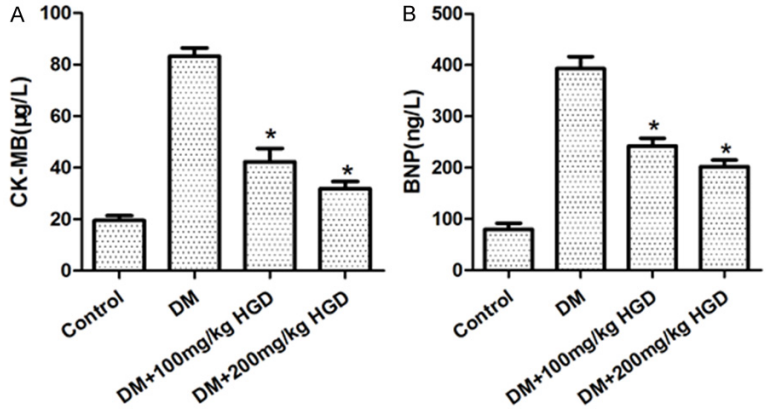


**Figure 3.** Effect of HGD on CK-MB and BNP. A: CK-MB; B: BNP. Data are means ± SD; *P<0.05 vs. DM group.

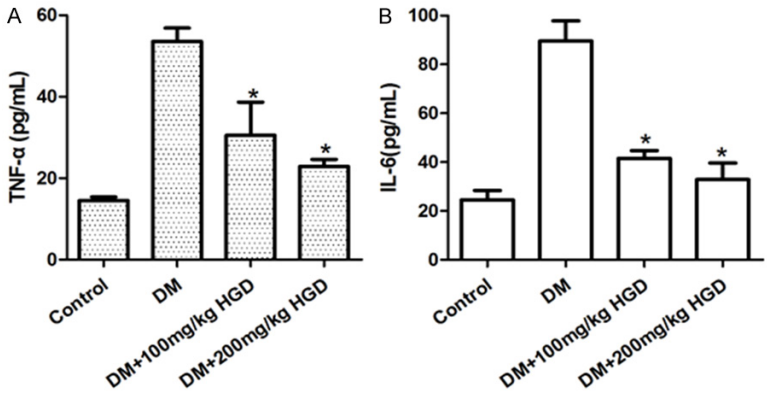


**Figure 4.** Effect of HGD on the expression of TNF-α and IL-6 in the diabetic rats. A: TNF-α; B: IL-6. Data are means ± SD; *P<0.05 vs. DM group.

*HGD decreases the levels of TNF-α and IL-6 in the plasma of diabetic rats*

Hyperglycemia is known to activate several cytokines, and cardiac inflammatory was reported to contribute to the development of DCM [11]. As we know that TNF-α and IL-6 are important maker of cardiac inflammatory, so we detected the change of TNF-α and IL-6 expression levels. As shown in **Figure 4**, there was a marked TNF-α and IL-6 decrease in the cytosol of diabetic hearts after treated with 100 mg/kg HGD or 200 mg/kg HGD, compared the DM group.

*HGD affects the expression of collagen I and MMP-2 in diabetic rats*

We also measure the expression of Collagen I and MMP-2, which are the markers of fibrosis. The mRNA and protein expression of Collagen I significantly higher in DM group when compared to the control group, however, there were significantly higher in the groups treated with HGD when compared to DM group. However, the expression of MMP-2 decreased significantly after treated with HGD, when compared to DM group (**Figure 5**).

*Effect of HGD on the Akt/NF-κb signaling pathway*

As showed in **Figure 6**, there was marked decrease in the phosphorylation of Akt and NF-κb in the diabetic hearts, compared to the control group. After treated with 00 mg/kg HGD or 200 mg/kg HGD, the phosphorylation of Akt increased in the myocardial tissues of diabetic mice. In addition, there was also marked increase in the NF-κb activation in the diabetic myocardium.

## Discussion

Diabete smellitus (DM) is a chronic disease caused by the interaction of heredity and environment [12]. Incidence and prevalence of diabetic cardiomyopathy are growing worldwide, about 65-70% of diabetic people have been died due to cardiac dysfunction. Diabetic cardiomyopathy (DCM), one of cause of morbidity and mortality in diabetic patients, is characterized by cardiac hypertrophy, cardiac fibrosis, decreased ventricular compliance, and diastolic and systolic dysfunction [13]. However, the development of DCM remains poorly understood and the underlying mechanisms have not yet been clearly elucidated.

STZ-induced DM is a well-established model for the study of diabetic cardiomyopathy [14]. In this study, we established the type 2 diabetes rat model by high-fat diet and low-dose streptozotocin (STZ), and treated with Huangqi gegen decoction (HGD), a potent antioxidant, to investigate for its ability to prevent diabetic cardio-

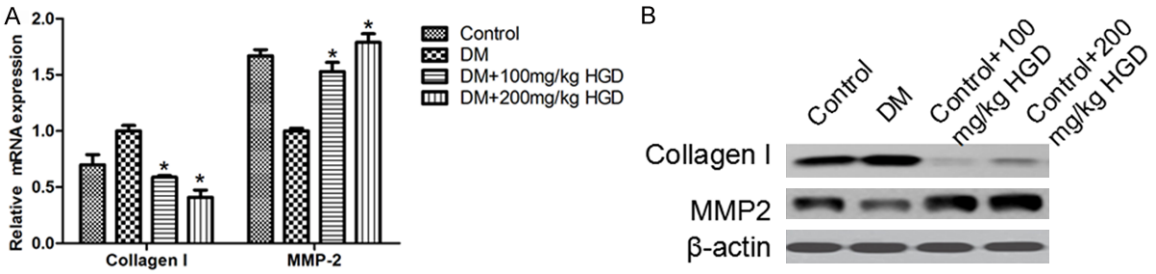


**Figure 5.** Effect of HGD on the mRNA and protein levels of TNF-α and IL-6 in the diabetic rats. A: mRNA expression of TNF-α and IL-6 were detected by Real-time PCR; B: Protein expression of TNF-α and IL-6 were detected by western blot. Data are means ± SD; *P<0.05 vs. DM group.

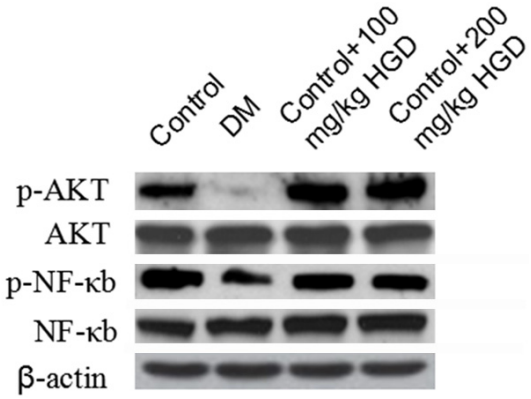


**Figure 6.** Effect of HGD on the Akt/NF-κb signaling pathway. Phosphorylation of Akt and NF-κb in myocardial tissues of rats were analyzed by western blot.

myopathy. We found that DCM rats showed that serum LVWI levels and HW/BW ratio were increased significantly compared to control rats (P<0.05), and showed significantly decreased blood LIWI levels and gained significantly higher BW after treated with HGD (P<0.05). Elevated serum BNP and CK-MB levels are the hallmark features of cardiac hypertrophy and have been employed to establish prognosis in determining myocardial injury [15]. Our study also found that BNP and CK-MB levels of DM rats decreased significantly after treated with HGD (P<0.05). These results indicated that HGD can improve diabetes-induced systolic dysfunction and myocardial injury.

Accumulating evidences suggest that inflammation plays a vital role in the initiation and progression of diabetic cardiomyopathy [16]. TNF-α and IL-6 are such cellular mediator that are capable of initiating and executing inflammatory response [17]. During the induction of inflammatory responses, TNF-α has a chemotactic function on neutrophils and monocytes and can cause their activation and degranulation. IL-6 could increases fibronectin expression and disturbs extracellular matrix dynamics to release inflammatory mediators. So we measure the TNF-α and IL-6 levels after treated with HGD. Our results showed that HGD could significantly attenuate the elevated levels of TNF-α and IL-6 in DM rats, thereby controlling the result of inflammation-mediated oxidative-nitrosative tissue injury.

Myocardial fibrosis is a crucial pathogenic factor in the progress of diabetic complications, including diabetic cardiomyopathy [14]. Accumulation of cardiac fibrosis can result in excessive production of collagen. MMPs were report to effectively involve in this turn-over by degrading collagens in cardiac tissue [18]. To assess the alterations of profibrotic mediators involved in myocardial fibrosis, myocardial levels of MMP2 and Collagen I was determined. Our results showed that HGD could significantly reduce the expression levels of Collagen I and markedly increase MMP-2 levels in DM rats. These results indicated that HGD could ameliorate cardiac fibrosis in streptozotocin-induced diabetic rats.

AKT was reported to regulate cardiovascular functions, including coronary angiogenesis, the growth of myocardial cells and cardiac systolic function [19]. NF-κB is a master transcription factor controlling the expression of a wide range of proinflammatory genes. Several studies have reported that AKT/NF-κB pathways are important inflammatory signaling pathways in cardiac remodeling of DCM. In our study, we found that the phosphorylation of Akt and NF-κB were significantly increased after treated with HGD, indicating that AKT/NF-κB may in-

volve in mediating the prevention of DCM by HGD.

In summary, our study demonstrate that Huangqi gegen decoction has a potential benefit in alleviation of diabetes associated cardiac fibrosis and inflammation by regulating the expression of TNF-α, IL-6, MMP2 and Collagen I. The effects of HGD are at least partly through the activation of the PI3K/Akt pathway.

## Disclosure of conflict of interest

None.

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