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ORIGINAL ARTICLE

Effects of Ginsenoside Rb1 on Serum Brain Natriuretic Peptide Level and Caspase-3 Protein Expression in Cardiomyocytes of Rats with Chronic Heart Failure

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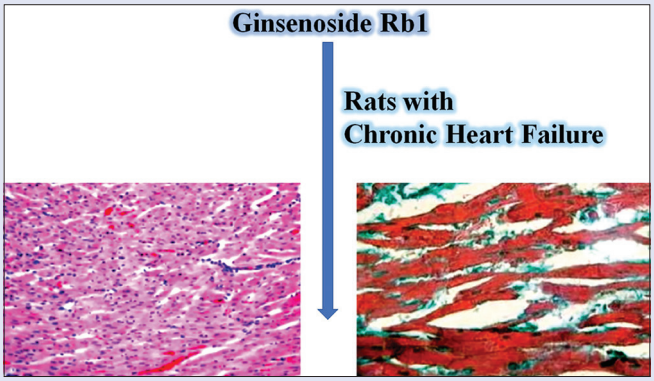
ABSTRACT

Context: Ginsenoside Rb1 is a representative ginsenoside, and caspase-3 is implicated in chronic heart failure (CHF). **Aim:** The aim of this study was to evaluate the effects of ginsenoside Rb1 on the level of brain natriuretic peptide (BNP) in serum and the expression of caspase-3 protein in the cardiomyocytes of CHF rats. **Subjects and Methods:** A total of 48 Wistar rats were divided into control, model, positive control (losartan), and ginsenoside Rb1 groups at random (n = 12). Abdominal aortic constriction was adopted for CHF modeling. Four weeks after surgery, ginsenoside Rb1 and losartan groups were intragastrically administered with 50 mg/kg ginsenoside Rb1 and 4.5 mg/kg losartan daily, respectively. Control and model groups were given equal volumes of distilled water. Cardiac function indices, electrocardiographic signals, BNP level, heart weight, body weight, heart-to-body weight ratio, myocardial pathological changes, and caspase-3 protein expression were compared. **Results:** In contrast to model group, heart rate, left ventricular end-diastolic pressure, BNP level, and caspase-3 protein expression of ginsenoside Rb1 and losartan groups notably dropped, whereas left ventricular systolic pressure and maximal rise/fall rate of the left ventricular pressure significantly rose ($P < 0.05$). The heart weight and heart-to-body weight ratio of ginsenoside Rb1 and losartan groups were evidently lower relative to those in the model group ($P < 0.05$). The ST segments of losartan and ginsenoside Rb1 groups fell after rise. Ginsenoside Rb1 inhibited focal cardiomyocyte necrosis and steatosis and relieved myocardial myofibrillar dissolution. It evidently decreased broken muscle bundles, as well as alleviated fibrosis and myocardial fibrosis. **Conclusion:** Ginsenoside Rb1 can improve the cardiac function of CHF rats, probably by inhibiting the apoptosis of cardiomyocytes.

Key words: Brain natriuretic peptide, cardiomyocyte, caspase-3, chronic heart failure, Ginsenoside Rb1, Wistar rats

SUMMARY

- Ginsenoside Rb1 inhibited focal cardiomyocyte necrosis and steatosis and relieved myocardial myofibrillar dissolution
- Ginsenoside Rb1 evidently decreased broken muscle bundles, as well as alleviated myocardial fibrosis
- Ginsenosides can improve the cardiac function of rats with chronic heart failure, probably by inhibiting cardiomyocyte apoptosis.



Abbreviations used: ±dP/dtmax: Maximal rise/fall rate of left ventricular pressure; BNP: Brain natriuretic peptide; CHF: Chronic heart failure; HE: H and e; HF: Heart failure; HR: Heart rate; LVEDP: Left ventricular end-diastolic pressure; LVSP: Left ventricular systolic pressure; TCM: Traditional Chinese medicine.

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INTRODUCTION

Heart failure (HF) is a common clinical syndrome that occurs when various harmful stimuli cause myocardial insufficiency, typified by high morbidity, hospitalization, and mortality rates.[1] In particular, people are prone to HF along with aging.[2] The core mechanism of onset of HF and progression is myocardial remodeling which further causes myocardial contraction and diastolic dysfunction. Although the prognosis of patients with HF has greatly improved, their mortality and hospitalization rates remain high.[3] Therefore, researchers have attempted to find new therapeutic drugs and targets.

In recent years, traditional Chinese medicine (TCM) has been successfully employed to treat HF. The TCM syndrome differentiation rule of HF shows that heart Qi and Yang deficiencies are the fundamental causes for HF. Thus, tonifying Qi plays a key role in HF treatment. Among TCM drugs, ginseng (*Panax ginseng* C. A. Meyer) is preferred because of its unique effects on reinforcing vital energy.[4] So far, over 50 types of ginsenosides have been identified from the root of ginseng.[5] Ginsenosides have clinical significance in the treatment of various cardiovascular diseases such as ischemic heart disease, arrhythmia, and HF.[6] These ginsenosides can be classified

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into oleanolic acid (e.g., Rh3), protopanaxadiol (e.g., Rb1, Rb2, and Rb3), and protopanaxatriol (e.g., Rg1 and Rg2).[7] Ginsenoside Rb1 is the representative protopanaxadiol ginsenoside with the highest content. It has well-known regulatory effects on the central nervous system, immune system, and tumors.[8,9] Moreover, ginsenoside Rb1 can suppress the apoptosis of cardiomyocytes, improve their tolerance to hypoxia, reduce their hypertrophy, regulate the PERK pathway, and affect the cell energy metabolism.[10,11] However, caspase-3 is a key protein of apoptosis, which is greatly expressed in cardiomyocytes in an HF animal model, suggesting that it is involved in the physiological process of HF.[12]

Therefore, in this study, we studied the effects of ginsenoside Rb1 on the cardiac function of rats with chronic HF (CHF) and detected the changes in the level of serum brain natriuretic peptide (BNP) and protein expression of myocardial caspase-3.

SUBJECTS AND METHODS

Experimental animals and drugs

A total of 48 8-week-old healthy Wistar rats of either gender were provided by Beijing Huakang Biotechnology Co. Ltd. (license number: SCXK [Beijing] 20089-0004). We obtained approval from the ethics committee of our hospital (Approval No. GUCM-201810003) to conduct animal experiments, and we took great care to minimize the suffering of animals.

Drugs and reagents

Ginsenoside Rb1 was purchased from Beijing Yingzena New Chemical Technology Research Institute (purity: 99.12%). Losartan potassium tablets were bought from Hangzhou Merck Sharp and Dohme Pharmaceutical Corp. (China). Anti-mouse caspase-3 monoclonal antibody was obtained from Cell Signaling (USA). BNP ELISA kit was provided by Shanghai Jiwei Biotechnology Co. Ltd. (China).

Animal feeding and grouping

After 1 week of feeding, the rats were randomly divided into four groups based on their body weights: blank control group, model group, positive control (losartan) group, and ginsenoside Rb1 group (n = 12; female/male ratio: 1/1). They were fed in individual cages using ordinary feed and tap water.

Modeling and drug treatment

The rats were intraperitoneally administered with 3.5 mL/kg chloral hydrate (10%) for anesthesia. Then, each rat was laid supinely on an operating table, shaved at a size of 3 cm × 5 cm in the middle of the abdomen, and disinfected with iodine.

A longitudinal incision was made 0.5 cm away from the left side of the abdominal midline to isolate the skin, muscle, peritoneum, and subcutaneous tissue, followed by adequate hemostasis. A part of the intestine, stomach, and spleen were pulled to the left side in the abdominal cavity and protected using saline gauze. The abdominal aorta segment was fully exposed. The abdominal aorta was freed at 0.5 cm on the branch of the right renal artery and a 7G needle (0.7 mm in diameter) was placed in parallel. The artery was ligated with a 0.7-mm needle with No. 0 suture, and then, the needle was removed, forming a lumen with about 70% stenosis.[13] For the sham operation group, the line was hung instead of ligation after the abdomen was opened and the other procedures were the same as those of the surgery group. Afterward, intraperitoneal organs were repositioned and 200,000 units of penicillin were injected intraperitoneally to prevent infection. The abdominal muscles and skin were thereafter sutured layer by layer. The rats were fully warmed after surgery. After being completely awake, they were returned to the cages and given antibiotics for 3 consecutive days. Four weeks after the surgery, the ginsenoside Rb1 and positive control groups were intragastrically administered with 50 mg/kg ginsenoside Rb1 and 4.5 mg/kg losartan daily, respectively. The control and model groups were administered with equal volumes of distilled water. All the rats were administered continuously for 8 weeks. Finally, the peripheral blood and heart tissue were collected.

Cardiac function determination

The rats were fasted for 12 h before surgery and were intraperitoneally injected with 3 mL/kg chloral hydrate (10%) for anesthesia. Each rat was laid supinely on an operating table and fixed. PowerLab data acquisition and analysis system were employed to collect electrocardiographic signals after connecting the right upper limb and lower limbs to the electrodes. The right carotid artery was dissected and a heparin-filled polyethylene catheter was connected to a clean pressure transducer. The catheter was marked in advance for the approximate length inside to avoid penetrating the heart. The distal end was ligated and the catheter was slowly inserted into the separated right carotid artery. When there was a sense of penetration under the hand and an apparently jagged wave line appeared in the blood pressure column of LabChart software (ADInstruments Inc, Colorado, US), the catheter had entered the left ventricle. Related indices, including heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), and maximal rise/fall rate of left ventricular pressure (±dP/dtmax), were recorded in the experimental process.

Measurement of serum brain natriuretic peptide level

Venous blood (1 mL) from rats was collected, and the serum was separated. The level of BNP was measured with an automatic biochemical analyzer according to kit's instructions.

Measurement of initial and final body weights, heart weight, and heart-to-body weight ratio

The body weights 24 h before the experiment (initial weight) and 24 h after the last administration (final weight) were measured by a balance. After 8 weeks of administration, the thoracic cavity was opened to observe the contraction of the heart and size. Then, the heart was removed, rinsed with precooled normal saline, and blotted up to measure the weight and to calculate the heart-to-body weight ratio.

Preparation and observation of myocardial sections

The rats were intraperitoneally administered with 3 mL/kg chloral hydrate (10%), and the thoracic cavity was dissected to disconnect the heart. The myocardial tissue was trimmed, rinsed with tap water overnight, soaked in gradient concentrations of ethanol and xylene for complete dehydration, and then embedded in liquid paraffin. The paraffin blocks were sectioned (4 μm in thickness).

H and e staining

The sections were deparaffinized and rehydrated with descending concentrations of xylene and ethanol solutions. The sections were rinsed with water, dyed with hematoxylin (5 min), placed in water, discriminated using 1% hydrochloric acid-ethanol (10 s), placed in water, dilute ammoniacal water added (60 s), rinsed with tap water to return to blue color (15 min), stained with eosin (3 min), placed in water, dewatered with ascending concentrations of ethanol and xylene solutions, transparentized, and finally mounted with neutral resin.

Masson staining

The deparaffinization and rehydration processes of sections were the same as those of H and e (HE) staining. Potassium dichromate was preheated at 52°C–60°C (about 1 h), and the sections were rinsed with tap water (5 min). Any pigmentation found under the microscope was removed using formalin. Then, the sections were stained with Masson solution (5 min), slightly rinsed with 0.2% freshly prepared aqueous acetic acid solution, differentiated using 5% phosphotungstic acid (5 min), rapidly rinsed (twice) with 0.2% aqueous acetic acid solution, dyed with bright green solution (5 min), quickly rinsed again by 0.2% aqueous acetic acid solution, dewatered by ascending concentrations of ethanol solutions, transparentized with xylene solutions at ascending concentrations, and subjected to mounting by neutral resin.

Detection of myocardial caspase-3 protein expression by Western blot

After myocardial tissue was cut into pieces, the cellular protein was taken out by the addition of lysis buffer and subjected to 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The product was thereafter transferred onto the nitrocellulose membrane which was blocked at room temperature (1 h) with 5% skimmed milk in Tris buffered saline/Tween (TBST) (150 mM NaCl, 20 mM Tris-HCl, and 0.1% Tween 20). Subsequent to rinsing with TBST (thrice, 15–20 min each time), the membrane was incubated with the primary antibody overnight at 4°C. Then, it was rinsed thrice with TBST (20 min/time) and incubated for 1 h with horseradish peroxidase-conjugated secondary antibodies at room temperature. Subsequently, the membrane was washed thrice with TBST (30 min each time). Enhanced chemiluminescence (ECL) solution (1:1) was utilized for 5-min color development. Image analysis software was used to the level of protein expression.

Statistical assessment

SPSS19.0 software was used to process the data obtained. Data were presented as mean ± standard deviation and subjected to normal distribution test. The data in nonnormal distribution were compared by the nonparametric test and those in normal distribution were compared using the variance homogeneity test. In the case of homogeneous variance, the intergroup comparison was implemented using one-way analysis of variance and the pairwise comparison was conducted using the least square difference method. In the case of heterogeneous variance, the intergroup comparison was conducted using Tamhane's T2 test and the pairwise comparison was performed using the Dunnett's method. $P < 0.05$ represented statistically significant differences.

RESULTS

Cardiac function indices

In contrast to the control group, HR and LVEDP of model group dramatically rose, but LVSP, +dP/dtmax, and −dP/dtmax evidently declined ($P < 0.05$). Relative to model group, HR and LVEDP of ginsenoside Rb1 and losartan groups significantly decreased, whereas LVSP, +dP/dtmax, and −dP/dtmax predominantly rose ($P < 0.05$) [Table 1]. Therefore, ginsenoside Rb1 evidently affected cardiac function indices.

Electrocardiographic changes

The ST segment of the model group was significantly elevated and was fused with the R wave. In contrast to the control group, the ST segments of losartan and ginsenoside Rb1 groups fell after rise, still being higher than the baseline level [Figure 1].

Serum brain natriuretic peptide levels

The level of BNP in serum of model group dramatically rose in contrast to the control group ($P < 0.05$). The serum BNP levels of ginsenoside Rb1 and losartan groups were predominantly lower than that of the model group ($P < 0.05$) [Figure 2].

Effects of ginsenoside Rb1 on heart weight, body weight, and heart-to-body weight ratio

All groups had similar final body weights. The heart of the model group was significantly enlarged with weak contraction. The heart weight and heart-to-body weight ratio of model group were evidently higher relative to those of the control group ($P < 0.05$). Ginsenoside Rb1 and losartan groups had evidently lower values than model group ($P < 0.05$) [Table 2].

Effects of ginsenoside Rb1 on myocardial pathology

HE staining showed that the myocardial tissue of normal rats had a clear texture. The myocardium was regularly shaped and arranged in parallel. The cell nucleus was clear, and the cells had no edema. The cardiomyocytes of the model group revealed edema and vacuolization and myocardial were fibers apparently ruptured. Ginsenoside Rb1 markedly inhibited focal cardiomyocyte necrosis and steatosis, relieved

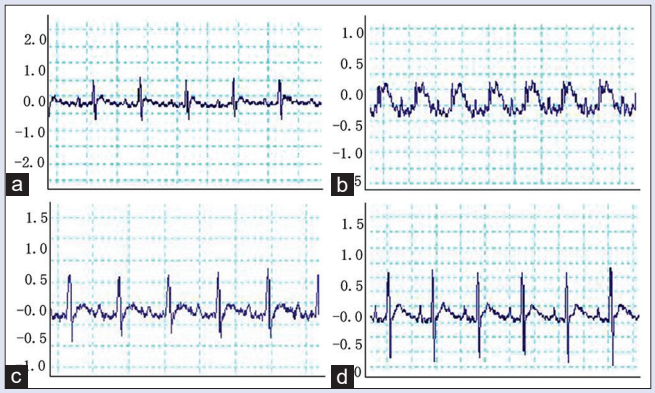

Figure 1: Electrocardiographic changes. (a) Control group; (b) Model group; (c) Ginsenoside Rb1 group; (d) Losartan group

Table 1: Cardiac function indices

Group	*n*	HR (bpm)	LVSP (mmHg)	LVEDP (mmHg)	+dP/dtmax (mmHg/s)	−dP/dtmax (mmHg/s)
Control	12	379±24	109.1±11.2	2.8±0.4	4588±213	4335±166
Model	12	490±23*	64.8±5.4*	20.4±2.4*	2105±176*	1843±145*
Ginsenoside Rb1	12	440±27#	86.2±6.3#	8.6±1.2#	3719±153#	3378±156#
Losartan	12	428±21#	87.8±5.8#	7.1±1.7#	3818±164#	3451±164#

*Compared with control group, $P<0.05$; #Compared with model group, $P<0.05$. HR: Heart rate; LVEDP: Left ventricular end-diastolic pressure; LVSP: Left ventricular systolic pressure; ±dP/dtmax: Maximal rise/fall rate of the left ventricular pressure

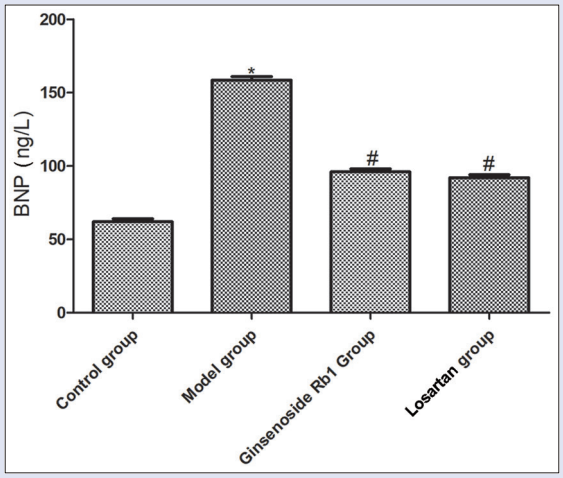

Figure 2: Serum BNP levels. *Compared with control group, $P < 0.05$; #compared with model group, $P < 0.05$. BNP: Brain natriuretic peptide

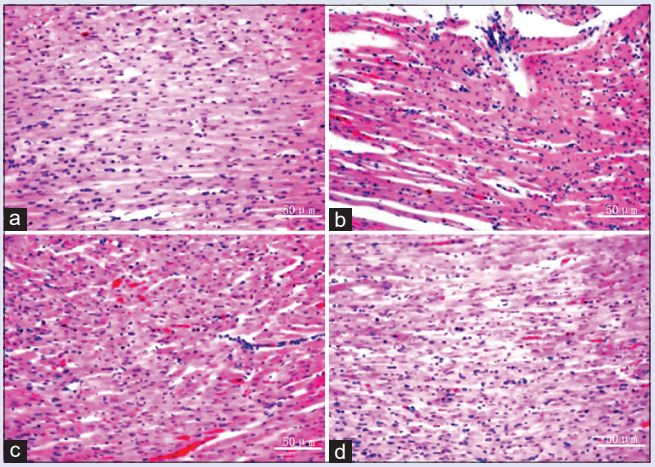

Figure 3: HE staining results. (a) Control group; (b) Model group; (c) Ginsenoside Rb1 group; (d) Losartan group. HE: H and e

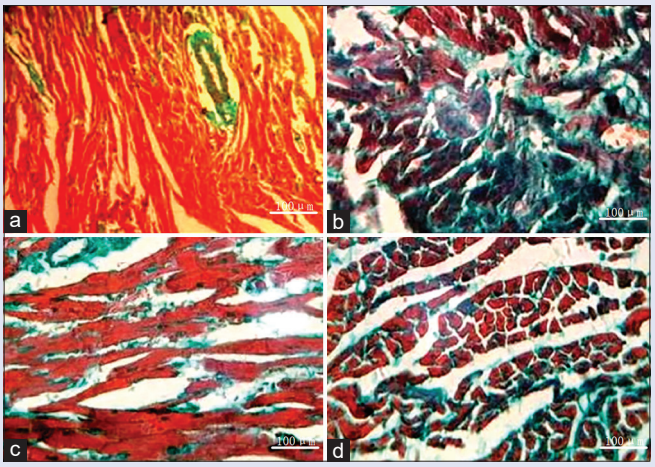

Figure 4: Masson staining results. (a) Control group; (b) Model group; (c) Ginsenoside Rb1 group; (d) Losartan group

myocardial myofibrillar dissolution, and inhibited interstitial capillary dilation [Figure 3]. Thus, ginsenoside Rb1 played a crucial role in mitigating the myocardial pathology of CHF rats.

Masson staining exhibited that ginsenoside Rb1 evidently decreased broken muscle bundles, as well as alleviated myocardial fibrosis [Figure 4].

Effects of ginsenoside Rb1 on myocardial caspase-3 protein expression

Model group had a dramatically higher myocardial expression of caspase-3 protein than that of the control group ($P < 0.05$). In contrast to the model group, the expressions of ginsenoside Rb1 and losartan groups markedly dropped ($P < 0.05$) [Figure 5].

DISCUSSION

CHF is an important cardiovascular event endangering human health.[14,15] At present, it is mainly treated with diuretics, angiotensin-converting enzyme inhibitors, β-blockers, and digitalis alone or in combination, but the side effects are also obvious.[16,17] According to the TCM theory, heart's Qi, Yin, and Yang are the fundamental causes for HF. Blood stasis, phlegm formation, and fluid retention are the pathological changes secondary to HF, forming a vicious circle.[18] Qi replenishment plays a vital role in the treatment of HF.[19] Among many TCM drugs, ginseng is given first priority because of its unique tonifying effects.

As one of the primary components of ginseng,[20] ginsenoside Rb1 can repress the apoptosis of cardiomyocytes caused by Ischemia/reperfusion injury (IRI).[21] In the model group, HR increased, and sufficient blood supply was provided by compensating the accelerated pulse. Nevertheless, most of them were ineffective pulses which aggravated ventricular hypertrophy. Moreover, electrocardiogram showed abnormalities, mainly manifested as ST-segment elevation, pathological change of Q wave, and abnormal HR. In summary, ginsenoside Rb1 effectively improved the cardiac function of CHF rats.

Ginsenoside Rb1 and losartan groups displayed remarkably lighter heart and lower heart-to-body weight ratio than that of model group. The former two groups had similar values, which suggests that ginsenoside Rb1 mitigated myocardial hypertrophy under pressure load. In addition, HE staining showed that ginsenoside Rb1 markedly relieved focal cardiomyocyte necrosis and steatosis, myocardial myofibrillar dissolution, and interstitial capillary dilation. Accordingly, ginsenoside Rb1 not only alleviated HF symptoms but it also protected the cardiac morphology.

Furthermore, ventricular remodeling is the primary cause for the death of patients with HF.[22,23] In this study, CHF rats underwent ventricular remodeling, as evidenced by ventricular hypertrophy and increase in the heart weight: body weight ratio. Masson staining revealed that ginsenoside Rb1 evidently decreased broken muscle bundles, as well as alleviated myocardial fibrosis. Possibly, ginsenoside Rb1 exerted therapeutic effects on CHF by relieving myocardial fibrosis, thereby suppressing ventricular remodeling and boosting cardiac function.

BNP, a polypeptide hormone comprising 32 amino acids, is primarily secreted and synthesized by ventricles. It has extremely high sensitivity and specificity for the diagnosis of HF. Serum BNP level can accurately reflect cardiac function in patients with CHF. At present, BNP is used as an independent diagnostic index for HF in clinical practice.[24,25] Herein, the serum BNP content of ginsenoside Rb1 group was evidently lower relative to that of model group, indicating that ginsenoside Rb1 significantly improved the heart function of rats with CHF.

In addition, caspases are expressed in cardiomyocytes, VSMCs, fibroblasts, and endothelial cells.[26,27] Of the 13 caspase family members,

Table 2: Effects of ginsenoside Rb1 on body weight, heart weight, and heart weight/body weight ratio

Group	*n*	Weight (g)		HW (mg)	HW (mg)/BW (g)
		Initial	Final		
Control	12	225.14±11.09	424.38±22.19	1074.28±113.29	2.53±0.23
Model	12	224.34±10.97	421.29±24.12	1674.23±109.24*	4.08±0.31*
Ginsenoside Rb1	12	226.45±11.24	419.78±22.39	1321.78±89.98#	3.11±0.29#
Losartan	12	223.48±11.25	420.19±21.33	1278.81±94.35#	2.93±0.31#

*Compared with control group, $P<0.05$; #Compared with model group, $P<0.05$. BW: Body weight; HW: Heart weight

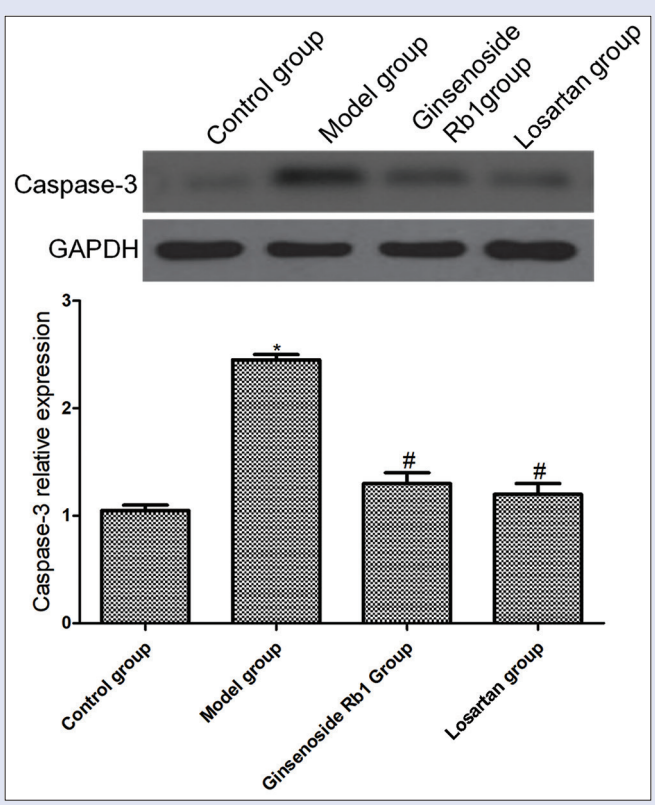

Figure 5: Effects of ginsenoside Rb1 on myocardial caspase-3 protein expression. *Compared with control group, $P < 0.05$; #Compared with model group, $P < 0.05$

caspase-3 is involved in cell apoptosis. For instance, myocardial caspase-3 in rabbits with HF is activated along with the apoptosis of cardiomyocytes.[28] Consistently, caspase-3 protein was expressed at an evidently elevated level in the myocardial tissue of model group in the current research. Subsequent to administration with ginsenoside Rb1, such expression decreased significantly, which remained higher relative to that of the control group. Hence, ginsenoside Rb1 may mitigate HF-induced myocardial remodeling by inhibiting cardiomyocyte apoptosis.

CONCLUSION

In summary, ginsenoside Rb1 can improve the cardiac function of rats with chronic HF, probably by suppressing apoptosis of cardiomyocytes.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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