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Original Article

Protective effects of glycyrrhetnic acid on a rat model of chronic mountain sickness

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Abstract: Objective: This study aims to explore the protective effects of glycyrrhetinic acid on the heart of rats with chronic mountain sickness (CMS). Methods: A CMS rat model was established and different doses of glycyrrhetinic acid were used to measure the pulmonary artery pressure (PAP) of rats. The serum vascular endothelial growth factor (VEGF), erythropoietin (EPO), C reactive protein (CRP), and apolipoprotein A1 (APO-A1) levels in rats were determined. The superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) activities in heart tissue were examined. Echocardiography was conducted to calculate right ventricular hypertrophy indices and to detect pathological changes. Results: Compared with normal rats, the rats in the model group have higher levels of PAP and serum VEGF, EPO, and CRP, lower levels of APO-A1, higher right ventricular hypertrophy indices, lower cardiac SOD and GSH-Px activities, and higher MDA level. Compared with those in the model group, the rats in all groups treated with different doses of glycyrrhetinic acid have lower level of PAP, lower serum VEGF, EPO, and CRP levels, higher level of APO-A1, lower right ventricular hypertrophy indices, higher cardiac SOD and GSH-Px activities, and lower MDA level. Conclusions: Glycyrrhetinic acid improves the heart injury in rats with chronic mountain sickness.

Keywords: Chronic mountain sickness, glycyrrhetinic acid, pulmonary hypertension, heart, hypoxia

Introduction

Chronic mountain sickness (CMS) is a clinical syndrome that often occurs in plateau populations (i.e., long-term residents in places above 2500 m in altitude). Its main characteristics include excessive erythrocyte proliferation, severe hypoxemia, and pulmonary hypertension. The condition may develop into pulmonary heart disease, leading to congestive heart failure in later stages. Approximately 140 million people worldwide live in plateau areas, and approximately 5%-10% of them are at risk of CMS [1-4]. Economic development and population growth may increase the migration of people toward plateau areas in the future. Therefore, the lack of effective treatment methods for CMS is an urgent problem that needs to be solved. At present, CMS treatment modalities include non-drug therapy, such as blood dilution [5] and long-term oxygen therapy [6], and drug therapy, including various drugs (i.e., acetazolamide [7], and nifedipine [8]) and traditional Chinese medicine (*Rhodiola*). These drugs can be used to alleviate CMS symptoms to some extent [9, 10].

Glycyrrhiza refers to the dry root and stem of leguminous plants *Glycyrrhiza uralensis*, *Glycyrrhiza inflata*, or *Glycyrrhiza glabra*. It is frequently used clinically due to its detoxification [11, 12], anti-inflammation [13], antitussive [14], and antiasthmatic effects [15]. The main components of glycyrrhiza are glycyrrhizic acid and glycyrrhetinic acid, which are acid triterpenoid saponins containing hydroxyl groups. Glycyrrhetinic acid is the aglycone of glycyrrhizic acid. Glycyrrhizic acid is hydrolyzed into glycyrrhetinic acid by intestinal bacteria after it is orally administered. Glycyrrhetinic acid is the

final substance with pharmacological effects *in vivo*. This acid presents several beneficial effects, such as antitumor, anti-inflammatory, antiviral, immune regulatory, and antioxidative properties [16-18]. Apolipoprotein A1 (Apo-A1) has coordinated anti-inflammatory effects, and C-reactive protein (CRP) is an inflammation marker. erythropoietin (EPO) plays a key role in the accumulation of red blood cells, and vascular endothelial growth factor (VEGF) plays a key role in the proliferation of vascular endothelial cells. These molecules are known to play important roles in cardiopulmonary regulation of individuals under high-altitude environment.

In this study, the effects of glycyrrhetinic acid intervention therapy in a CMS rat model were investigated, levels of pulmonary artery pressure (PAP); VEGF, EPO, CRP, and APO-A1; and cardiac superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px) activities were measured. Changes in echocardiography, right ventricular hypertrophy index, and pathology were monitored. The main purpose of this study was to explore the protective effects of glycyrrhetinic acid on the heart of CMS rats.

Material and methods

Instruments

The following instruments were used: Multiskan Go Multiskan Spectrum (Thermo Fisher Scientific, USA), 1575 Microporous Thermo Scientific Plate Washer (Bio-Rad Medical Products Co., Ltd., USA), 3-18K high-speed cryogenic centrifuge (Siemens AG, German), SAR-830 Small Animal Ventilator (Shanghai Yuyan Scientific Instrument Co., Ltd., China), Special Northwest Artificial Environmental and Experimental Chamber (Key Medicine Laboratory of Special Environment, Xinjiang), HDII XE Color Doppler Ultrasound Diagnostic Instrument (Philips Electronics NV, Netherlands), and JEOL1230 transmission electron microscope (JEOL Ltd., Japan).

Reagents

The following reagents were used in this study: nifedipine tablets (Shanxi Yun Peng Pharmaceutical Co., Ltd., Batch No. C120304), glycyrrhetinic acid (Shanghai Yuanye Biotechnology Co., Ltd.), rat EPO ELISA kit (Batch No. AD20180708, Abnova Corporation, China), rat C Reactive Protein ELISA kit (Batch No. GR3184775-11, Abcam Corporation, China), rat VEGF kit (Batch No. 201807, Anhui Joyee Biotechnology Co., Ltd.), rat Apo-A1 kit (Batch No. AD20180708, Andy Gene Biotechnology Co., Ltd., China), rat SOD ELISA kit (Batch No. 20180920, Nanjing JianCheng Bioengineering Institute), rat MDA ELISA kit (Batch No. 20180919, Nanjing JianCheng Bioengineering Institute), and GSH-Px ELISA kit (Batch No. 20180911, Nanjing JianCheng Bioengineering Institute).

Animals

Sixty SD rats weighing 180 ± 20 g were purchased from the Animal Center of Xinjiang Medical University (Animal License No. SCXK [xin] 2011-0004).

Ethics approval and consent to participate

This study was approved by the First Affiliated Hospital of Xinjiang Medical University (approval number: IACUC-20160218009).

Establishment of the CMS model

Fifty SD rats were randomly chosen and placed in a special northwest artificial environmental and experimental chamber to simulate a plateau environment at 5000 m altitude for establishing CMS. The rats were placed in the chamber 22 h per day for 30 d to enable continuous modeling. The remaining 10 SD rats were placed in a regular environment and reared in the Animal Center of Xinjiang Medical University.

Grouping and administration methods

Fifty SD rats with CMS were randomly divided into the model group (MG), nifedipine group (Nif), high-dose glycyrrhetinic acid group (GA-H), moderate-dose glycyrrhetinic acid group (GA-M), and low-dose glycyrrhetinic acid group (GA-L) and given the following interventions: ① MG: 4 mL/kg/d distilled water administered into the stomach for 15 d; ② Nif: nifedipine administered at a dosage of 2.7 mg/kg/d for 15 d; ③ GA-H: 80 mg/kg/d of glycyrrhetinic acid administered into the stomach for 15 d; ④ GA-M: 40 mg/kg/d of glycyrrhetinic acid administered into the stomach for 15 d; and ⑤ GA-L: 20 mg/kg/d of glycyrrhetinic acid administered into the stomach for 15 d.

Ten SD rats in a regular environment were included as the control group (CG) and received the following intervention: ⑥ CG: 4 mL/kg/d distilled water administered into the stomach for 15 d.

2D echocardiography

A mixture of 1 mL of atropine (0.5 mg), 2 mL of diazepam (10 mg), and 2 mL of ketamine (100 mg) were diluted to 10 mL. The rats were anesthetized in 0.75 mL/100 g and then fixed to the operating table. The pectoris anterior of the rats was dehaired, A color Doppler ultrasound was used to detect the right ventricular diameter, right ventricular anterior wall thickness, right ventricular outflow tract, inner diameter of the left ventricular systolic and diastolic periods, interventricular septal thickness, and ejection fraction. The left ventricular function parameters and ventricular motion were measured by using M-mode echocardiography on the long-axis section of the left ventricle near the sternum.

Determination of PAP

The skin from the neck and chest was cut after 2D echocardiography of the rat heart. The chest was opened from the midline of the sternum to expose the lungs and heart. A catheter was inserted from the right vena jugularis externa to the right atrium, into the ventriculus dexter, and then into the pulmonary artery. PAP waveforms were subsequently observed. The other end of the needle was connected to a pressure transducer, and pressure change data were recorded with a biosignal recorder.

Serum markers

Blood was obtained from the abdominal aorta and allowed to stand at room temperature for 30 min. After low-temperature centrifugation for 20 min (3000 rpm), the supernatant fluid was obtained and separately packed for cryopreservation in a refrigerator at -80°C. Following the instructions of the relevant kits, the supernatant fluid of rat serum was used to determine the CRP, Apo-A1, EPO, VEGF, and other indices.

Detection of right ventricular hypertrophy index

Blood was obtained from the abdominal aorta, and the heart was collected. After confirming the structure of the heart, the atrium, auricle, and other tissues around the organ were cut off. Starting from the outlet of the pulmonary artery, the right ventricular tissue (RV) was cut off along the edge of the ventricular septum. The remainder was the left ventricle and ventricular septum tissue (LV+S). Excess blood was drained with filter paper. A precision balance was used to weigh the tissues, and the right ventricular hypertrophy index (RVHI) was calculated from the formula (mass RV)/[mass (LV+S)].

Changes in heart pathology

The ventriculus dexter was obtained and fixed in 10% formaldehyde solution. The specimens were placed in 80% ethanol, 95% ethanol, and absolute alcohol for dehydration and then soaked in dimethylbenzene to render them transparent. The samples were then embedded in paraffin, cut into 5 μm slices, dewaxed with dimethylbenzene, and dehydrated with gradient ethanol. The samples were dyed with hematoxylin, differentiated with 1% ethanol hydrochloride, blued with 1% ammonia solution, stained with eosin, dehydrated with gradient ethanol, and covered with neutral gum.

Ultrastructural examination of the heart

Tissues were cut into 1 mm^3 pieces, placed in 2.5% glutaraldehyde, and fixed at 4°C for 24 h. The tissue pieces were rinsed with 2% sodium dimethylarsenate buffer, fixed at 4°C with 1% osmic acid containing 1% potassium ferrocyanide, and washed with double-steamed water. The samples were dehydrated using gradient ethanol and embedded and polymerized using epoxy resin (Epon812). The embedded tissue pieces were trimmed and cut into 60-70 nm slices. The samples were then stained with uranium acetate and lead citrate for observation through transmission electron microscopy (TEM).

Statistical method

SPSS19.0 was used for statistical analysis. Data were presented as mean ± standard deviation ($\bar{x} \pm s$) and were subjected to the normality test and homogeneity test of variance. The mean values between groups of data with normal distributions were compared through one-way ANOVA, and $\alpha = 0.05$ was considered to indicate statistical significance.

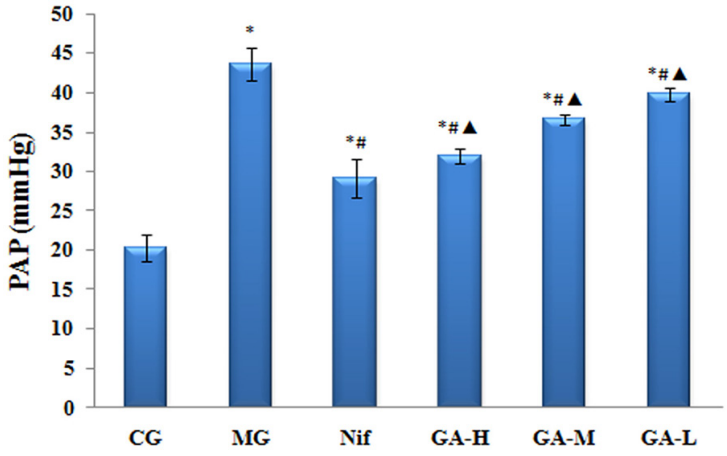


Figure 1. Effect of glycyrrhetinic acid on PAP. Note: Compared with CG, $^{*}P < 0.05$; compared with MG, $^{\#}P < 0.05$; compared with Nif, $^{\blacktriangle}P < 0.05$.

Results

Effect of glycyrrhetinic acid on PAP

The PAP of MG, Nif, GA-L, GA-M, and GA-H rats increased compared with that of CG rats, and the differences were statistically significant ($P < 0.05$). Compared with that of the model group, the PAP of Nif, GA-L, GA-M, and GA-H rats decreased significantly ($P < 0.05$). Compared with that of the Nif group, the PAP of rats in the GA-L, GA-M, and GA-H groups significantly increased to different degrees ($P < 0.05$) (**Figure 1**).

2D echocardiography

Compared with that of control rats, the inner diameters of the left and right (transverse diameter) atria of MG rats increased. The inner diameter of the left ventricular systolic and diastolic periods decreased. The changes in right ventricular diameter were not evident, and the anterior wall of the right ventricle was markedly thickened. The right ventricular outflow tract widened, and the ejection fraction decreased. Thus, the CMS rat model was successfully established.

Compared with that of MG rats, the inner diameter of the left atrium of GA-L, GA-M, and GA-H rats decreased. The inner diameter (transverse diameter) of the right atrium decreased in the GA-M and GA-H groups relative to that of the MG rats. No significant change was observed in the inner diameters of the left ventricle in the GA-L, GA-M, and GA-H groups in the systolic and diastolic periods. The inner diameter of the right ventricle was unchanged. The thickness of the anterior wall of the right ventricle of rats in the GA-M group decreased. The change in the outlet tract of the right ventricle of rats in the GA-L, GA-M, and GA-H groups was insignificant, and the ejection fraction increased (**Figure 2**).

Effect of glycyrrhetinic acid on the RVHI

Compared with that of CG rats, the RVHI of rats in the MG, Nif, GA-L, GA-M, and GA-H groups significantly increased ($P < 0.05$). Compared with that of MG rats, the RVHI of rats in the Nif, GA-L, GA-M, and GA-H groups significantly decreased ($P < 0.05$). Compared with that of Nif rats, the RVHI of rats in the GA-H and GA-M groups significantly decreased ($P < 0.05$) (**Figure 3**).

Effect of glycyrrhetinic acid on cardiac SOD, GSH-Px, and MDA activities

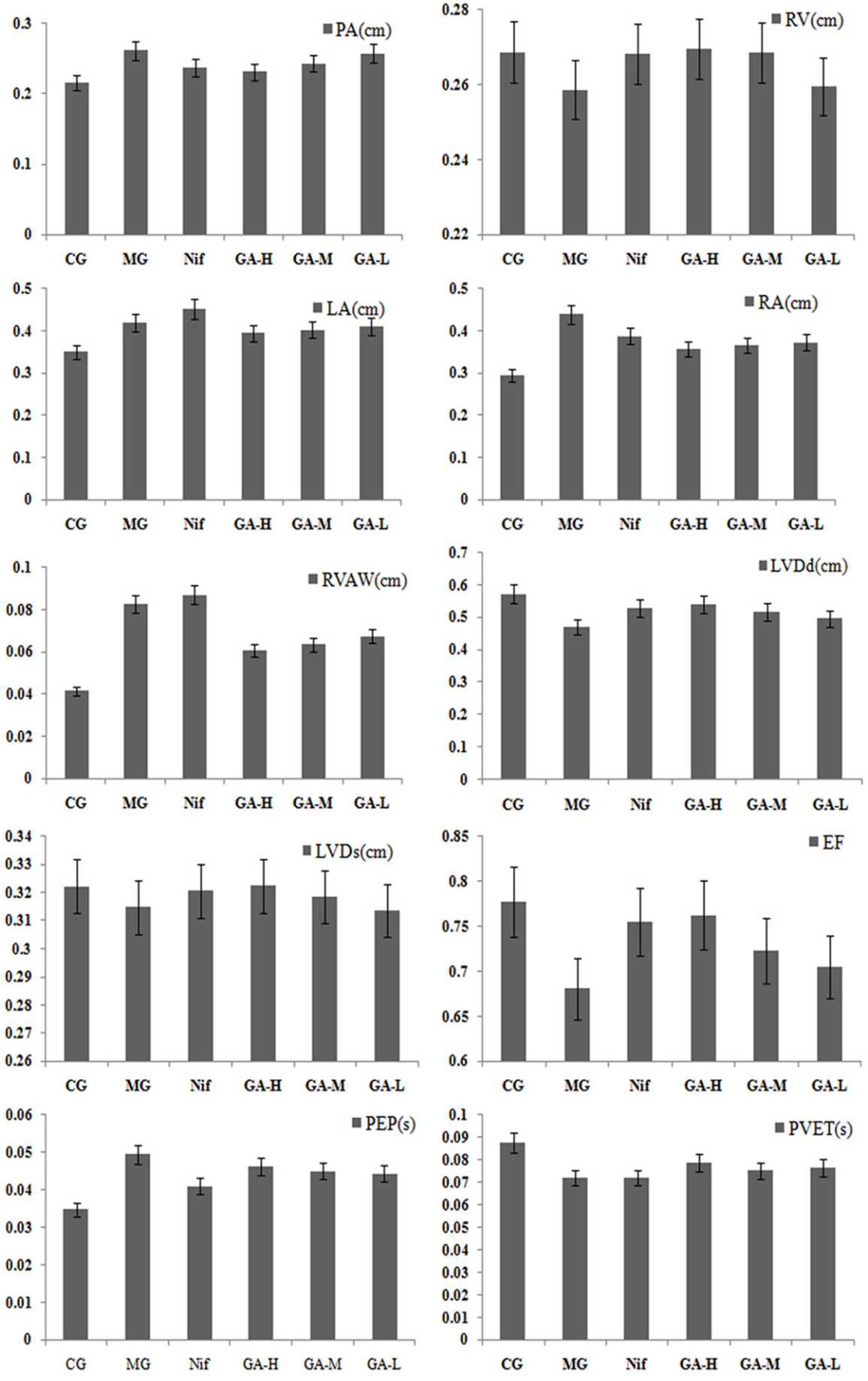
Compared with those in the CG, the cardiac SOD and GSH-Px activities of MG, Nif, GA-L, GA-M, and GA-H rats decreased. By contrast, the MDA contents increased. The differences were statistically significant ($P < 0.05$).

Compared with those of MG rats, the cardiac SOD and GSH-Px activities of rats in the Nif, GA-L, GA-M, and GA-H groups increased. The MDA contents decreased. The differences were statistically significant ($P < 0.05$).

Compared with those of Nif rats, the cardiac SOD and GSH-Px activities of rats in the GA-L, GA-M, and GA-H groups increased, and the MDA contents decreased. The differences were statistically significant ($P < 0.05$) (**Figure 4**).

Cardiological examination

Biopsy of the heart tissues of CG rats was conducted. No abnormal exudates attached to the epicardium and no thickening due to mechanization were observed under low and high magnification. In this group, myocardial fibers were arranged regularly with clear transverse lines and no abnormal necrosis or deformation was found. Myocardial cells were clearly stained


PA(cm)
RV(cm)
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CG
MG
Nif
GA-H
GA-M
GA-L

Figure 2. 2D echocardiography. PA: Pulmonary Artery; LA: Left Atrium; RA: Right Atrium; RV: Right Ventricle; RVAW: Right Ventricular Anterior Wall; LVDd: Left Ventricular End Diastolic Dimension; LVDs: Left Ventricular End-Systolic Dimension; EF: Ejection Fraction; PEP: Pre-ejection period; PVET: Right Ventricular Ejection Time.

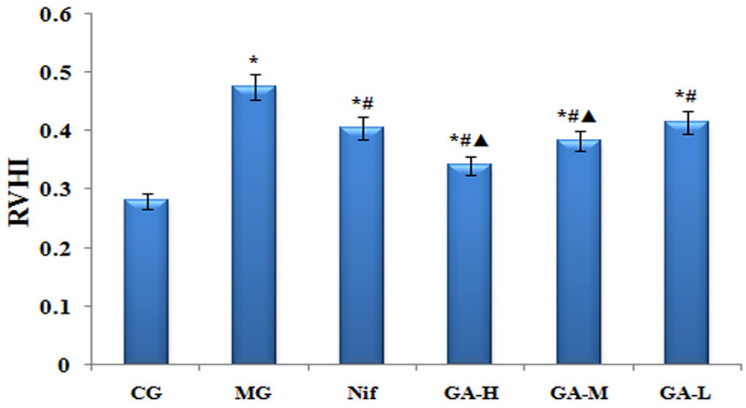

Figure 3. Effect of glycyrrhetinic acid on the right ventricular hypertrophy index. Note: Compared with CG, $^{*}P < 0.05$; compared with MG, $^{\#}P < 0.05$; compared with Nif, $^{\blacktriangle}P < 0.05$.

with dense nuclei. Congestion and edema were not observed in the myocardial interstitial vessels. Significant inflammatory cell infiltration was absent, and the myocardial structure was normal.

Biopsy of the heart tissues of MG rats was conducted. Under a low-power lens, subepicardial congestion was obvious, and myocardial fibers were disordered with unclear transverse lines. Deformations were observed. Eosinophilic lesions of some myocardial fibers, granular degeneration of the cytoplasm, marked congestion of some myocardial interstitial vessels, and infiltration of inflammatory cells were observed under a high-power lens.

Biopsy of the heart tissues of Nif rats was conducted. Under a low-power lens, no obvious exudates attached to the epicardium were observed, and the myocardial fibers were irregularly arranged with unclear transverse lines. Eosinophilic lesions of some myocardial fibers, mild granular degeneration of the cytoplasm, marked congestion of some myocardial interstitial vessels, and infiltration of inflammatory cells were observed under a high-power lens.

Biopsy of the heart tissues of GA-H rats was conducted. Under a low-power lens, mild subepicardial congestion was obvious, and myocardial fibers were irregularly arranged with unclear transverse lines. Under a high-power lens, granular degeneration of the myocardial fibers, infiltration of a small number of eosinophilic lesion cells in myocardial fibers, mild dilatation, congestion of some myocardial interstitial vessels, and infiltration of a small number of inflammatory cells were observed.

Biopsy of the heart tissues of GA-M rats was conducted. Under a low-power lens, no abnormal exudates attached to the epicardium were found, and the myocardial fibers were arranged regularly with clear transverse lines. Under a high-power lens, mild granular degeneration of myocardial fibers, occasional eosinophilic lesion cells, no dilatation and hemorrhage of myocardial interstitial vessels, and no obvious inflammatory cell infiltration were observed. The degree of lesions in these rats was slightly milder than that in GA-H rats.

Biopsy of the heart tissues of GA-L rats was conducted. Under a low-power lens, the epicardium was thin with no obvious exudates attached, and the myocardial fibers were arranged regularly with clear transverse lines; however, slight deformations were observed. Under a high-power lens, the myocardial fibers showed mild granular changes, no obvious eosinophilic infiltration, slight dilatation and hemorrhage of some myocardial interstitial vessels, and no obvious inflammatory cell infiltration. The degree of lesions in this group was slightly milder than that in the GA-M and GA-H rats (**Figure 5**).

TEM heart examination

The myolemma of cardiomyocytes in CG rats was smooth, and the myofibrils were arranged neatly with clear and neat Z lines. A large number of mitochondria were observed between myofibrils, and the ridges were abundant and arranged neatly.

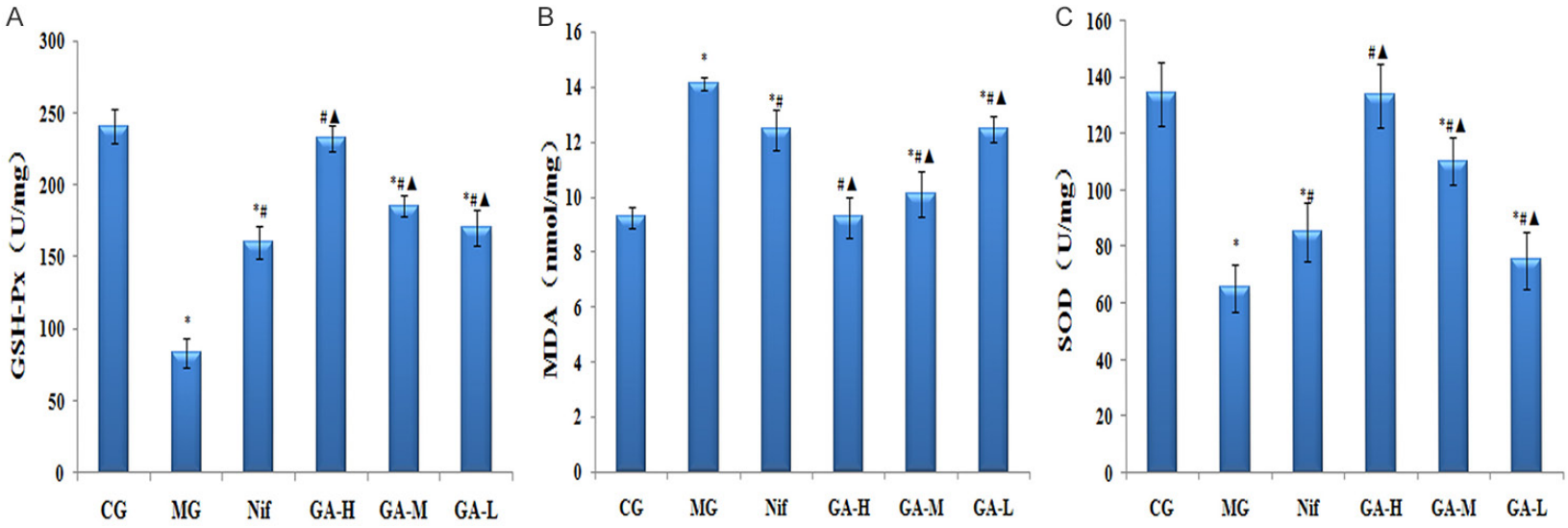

Figure 4. Effect of glycyrrhetinic acid on cardiac SOD, GSH-Px, and MDA activities. Note: Compared with CG, $^{*}P < 0.05$; compared with MG, $^{\#}P < 0.05$; compared with Nif, $^{\blacktriangle}P < 0.05$.

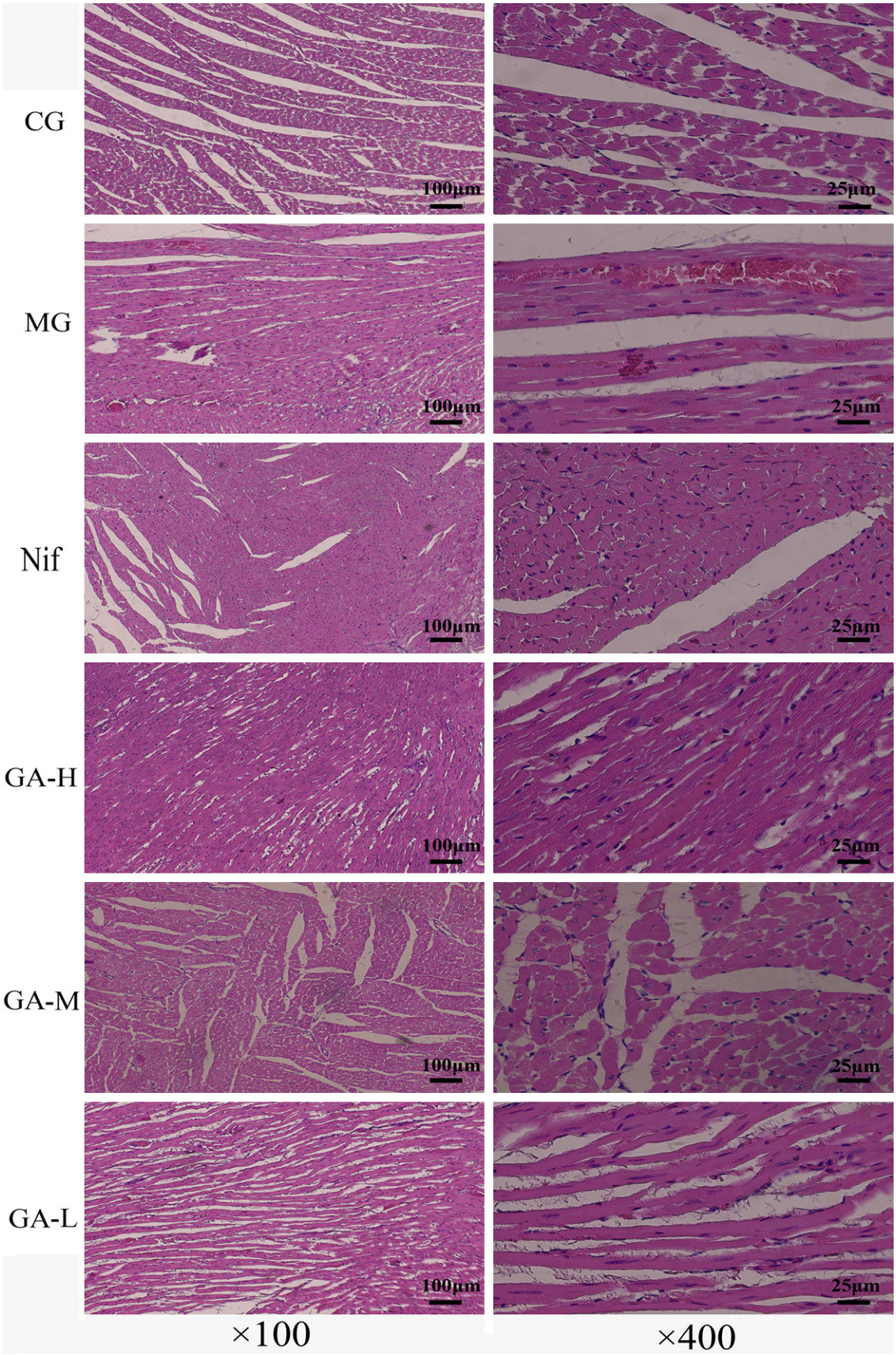

Figure 5. Cardiological examination.

The myocardial myofibrils of MG rats were obviously disordered. The sarcomeres were unclear, the mitochondria were proliferated and spindle shaped, and the cristae were fractured. The local cristae of mitochondria dissolved into flocs.

The myocardial myofibrils of Nif rats were arranged neatly with a small amount of lipid droplets. The endoplasmic reticulum was slightly expanded, and the mitochondrial cristae and matrix were focal and disordered. Mild edema surrounded the mitochondria.

The myocardial myofibrils of GA-L rats were disordered, the endoplasmic reticulum was remarkably expanded, and the amount of glycogen observed was high. The mitochondrial cristae were focally blurred and disordered, and peripheral edema was found.

The myocardial myofibrils of GA-M rats were arranged neatly with slightly uneven Z lines. The endoplasmic reticulum was slightly expanded, and some mitochondrial cristae dissolved into flocs.

The myocardial myofibrils of GA-H rats were arranged neatly with slightly uneven Z lines. The mitochondrial cristae were focally blurred and disordered (**Figure 6**).

Examination of rat serum

Compared with that of the CG, the serum CRP and EPO contents of rats in the MG, Nif, GA-L, GA-M, and GA-H groups were significantly higher ($P < 0.05$). Compared with that of MG rats, the serum CRP and EPO contents of rats in the Nif, GA-L, GA-M, and GA-H groups were significantly lower ($P < 0.05$). Compared with that of Nif rats, the serum CRP contents of rats in the GA-L, GA-M, and GA-H groups were significantly lower. Interestingly, the serum EPO contents of rats in the GA-L and GA-M groups were significantly high ($P < 0.05$) (**Figure 7**).

Compared with that of the control rats, the serum Apo-A1 contents of rats in the MG, Nif, GA-L, GA-M, and GA-H groups were significantly lower ($P < 0.05$). Compared with that of MG rats, the serum Apo-A1 contents of rats in the Nif, GA-L, GA-M, and GA-H groups were significantly higher ($P < 0.05$). Compared with that of Nif rats, the serum Apo-A1 contents of rats in the GA-H and GA-L groups were lower whereas those of rats in the GA-M group were significantly higher ($P < 0.05$).

Compared with that of the CG, the serum VEGF contents of rats in the MG and Nif groups were higher but those of rats in the GA-H and GA-M groups were significantly lower ($P < 0.05$). Compared with that of MG rats, the serum VEGF content of rats in the Nif, GA-L, GA-M, and GA-H groups were significantly lower ($P < 0.05$). Compared with that of Nif rats, the serum VEGF content of rats in GA-L, GA-M, and GA-H groups were significantly lower ($P < 0.05$) (**Figure 8**).

Discussion

The main pathological features of CMS are hypoxemia, polycythemia, and pulmonary hypertension. Pulmonary hypertension is the most serious and frequent symptom of the syndrome. High-altitude hypoxia causes pulmonary vasoconstriction and high blood flow resistance [19]. Pulmonary vasoconstriction is the main factor in the early stages of high-altitude pulmonary hypertension, Prolongation of hypoxia leads to pulmonary vascular remodeling. PAP increases as the remodeled pulmonary vessels become thick and have narrow lumen. The experimental results showed that the PAP of CMS rats remarkably increases. PAP decreases to some extent after intervention with different doses of glycyrrhetinic acid, thereby suggesting that glycyrrhetinic acid can decrease PAP to a certain extent.

Reactive oxygen species (ROS) are highly reactive substances that are rich in aerobic elements [20]. ROS production and consumption are usually balanced in the body. Metabolic disorders may arise when an organism is stimulated by internal or external factors, resulting in excessive ROS production or decreased ROS consumption. Excess ROS can cause oxidative damage to cell membranes, leading to oxidative stress damage in many tissues. Hypoxia may occur at high-altitude environment to induce ROS overload, thereby leading to hypoxic pulmonary hypertension [21]. When an organism is exposed to a high-altitude environment, its blood oxygen content decreases, and the organism enters an oxidative stress state. The contents of oxidative damage products, including methionine sulfoxide and MDA, increase, and the activities of SOD, GSH-Px, and other endogenous antioxidant enzymes

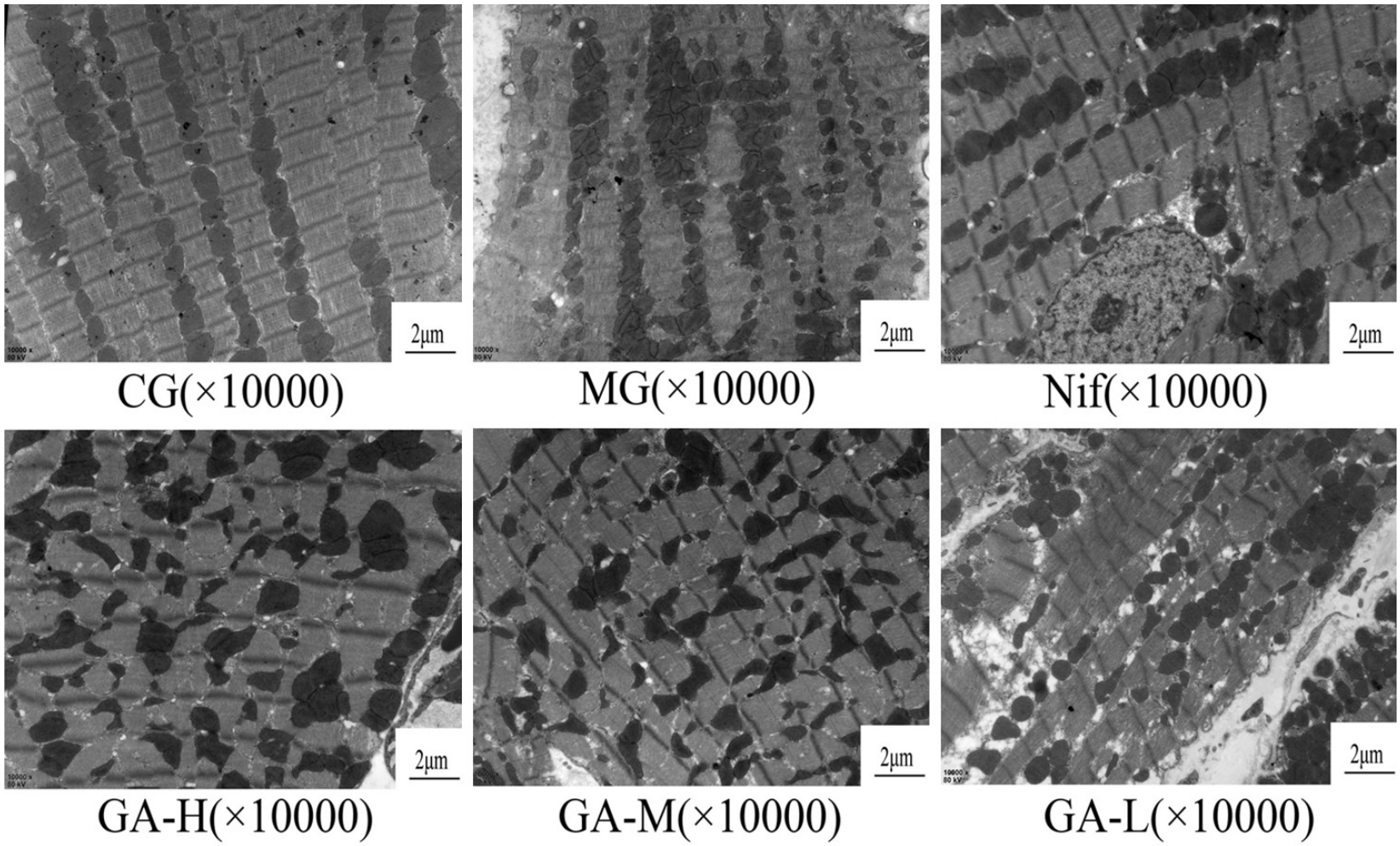

Figure 6. TEM heart examination.

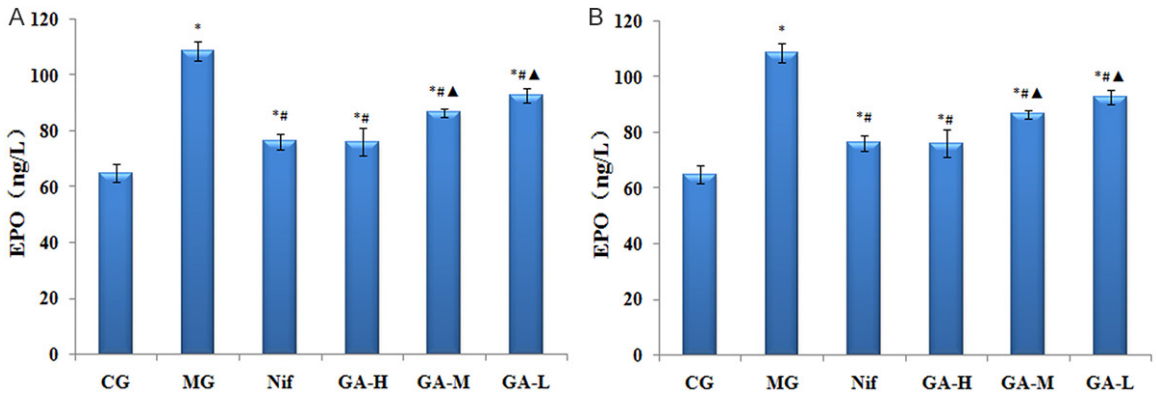

Figure 7. Results of CRP and EPO contents in serum. Note: Compared with CG, $^{*}P < 0.05$; compared with MG, $^{\#}P < 0.05$; compared with Nif, $^{\blacktriangle}P < 0.05$.

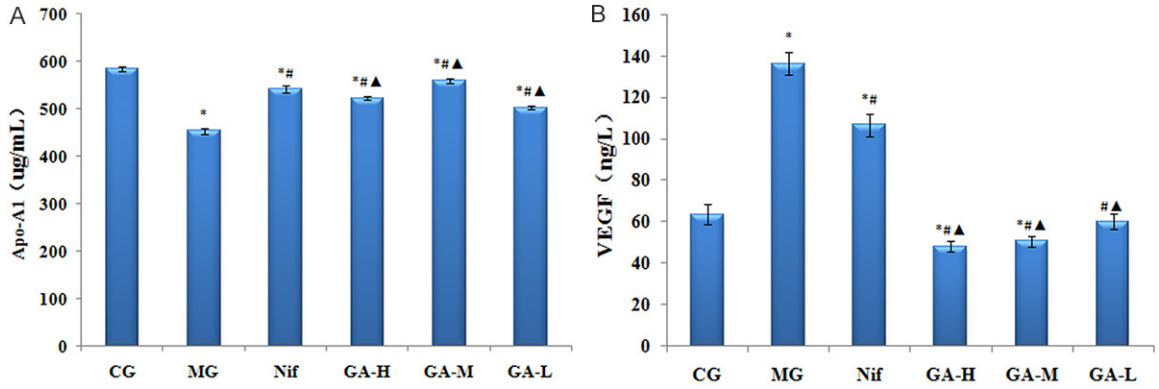

Figure 8. Results of VEGF and APO-A1 contents in serum. Note: Compared with CG, $^{*}P < 0.05$; compared with MG, $^{\#}P < 0.05$; compared with Nif, $^{\blacktriangle}P < 0.05$.

decrease. Our results showed that cardiac SOD and GSH-Px activities in CMS rats decreased, and MDA contents increased. These results indicate that CMS rats are in an oxidative stress state and may have heart damage. After glycyrrhetinic acid intervention, cardiac SOD and GSH-Px activities in CMS rats increased, and MDA contents decreased. Thus, glycyrrhetinic acid can alleviate oxidative damage to the heart and has a certain antioxidative effect. Pathological examination reveals that glycyrrhetinic acid can improve cardiac injury in CMS rats.

Chronic hypoxia can directly stimulate systemic inflammation in an organism residing in a high-altitude environment for a long time. CRP is an important inflammatory mediator; its level can be used to determine the degree of tissue damage and as a risk factor for cardiovascular disease [22]. CRP can directly damage vascular endothelial cells, promote ET-1 secretion, reduce NOS levels, and induce endothelial dysfunction and pulmonary vasospasm, thereby participating in the formation of HAPH [23]. Apo-A1 has anti-inflammatory and antioxidative effects. During inflammation, increased secretion of inflammatory cytokines, such as IL-1beta, IL-6, and TNF-alpha, reduces the Apo-A1 level in the plasma [24]. Decreases in Apo-A1 level lead to the uncontrolled release of these cytokines, thereby forming a vicious cycle that causes multiple organ dysfunction. The experimental results show increase in serum CRP and decrease in serum APO-A1 in CMS rats after treatment with glycyrrhetinic acid. This finding indicates that glycyrrhetinic acid has a certain anti-inflammatory effect.

EPO, an endogenous glycoprotein hormone in organisms, can promote the production of red blood cells. When an organism has lived in a

high-altitude environment for a long time, hypoxia can stimulate the production of large amounts of EPO. After combination with the target organ, the JAK2 and STAT-5 signal transduction pathways may be activated to induce red blood cell proliferation, resulting in increased blood viscosity, microcirculation disturbance, thrombosis, and extensive organ damage [25, 26]. Previous studies proved that VEGF can promote endothelial cell proliferation and induce angiogenesis [27]. Therefore, myocardial tissue damage induced by high-altitude hypoxia can stimulate the secretion of VEGF, leading to angiogenesis and pathological changes, such as myocardial fibrosis, myocardial interstitial vasodilation, and congestion. EPO, as a cytoprotective factor, can promote the production of new blood vessels by increasing the expression of VEGF under hypoxia and improve cardiac function. Thus, serum EPO and VEGF contents increased in CMS rats. After administration of GA, serum EPO and VEGF levels in the serum decreased. These findings reveal that chronic hypoxia stimulates EPO and VEGF production, leading to cardiac injury. After administration of GA, serum EPO and VEGF levels decreased, and heart damage was slightly alleviated.

In conclusion, glycyrrhetinic acid can significantly improve cardiac injury in CMS rats. Glycyrrhetinic acid may decrease the right ventricular afterload and improve right ventricular hypertrophy in CMS rats by reducing PAP. This acid can also alleviate heart injury from hypoxia-mediated inflammatory mediators and oxidative stress.

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Disclosure of conflict of interest

None.

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