Ginsenoside Rb1 attenuates doxorubicin induced cardiotoxicity by suppressing autophagy and Nrf2 induced ferroptosis

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Abstract

Ginsenoside Rb1 (Rb1), an active component isolated from traditional Chinese medicine Ginseng, is beneficial to many cardiovascular diseases. However, whether it can protect against doxorubicin induced cardiotoxicity (DIC) is not clear yet. In this study, we aimed to investigate the role of Rb1 in DIC. Mice were injected with a single dose of doxorubicin (20mg/kg) to induce acute cardiotoxicity. Rb1 was given daily gavage to mice for 7 days. Changes in cardiac function, myocardium histopathology, oxidative stress, cardiomyocyte mitochondrion morphology as well as autophagy and ferroptosis pathway were studied to evaluate Rb1’s function on DIC. Meanwhile, RNA-seq analysis was performed to explore the potential underline molecular mechanism involved in Rb1’s function on DIC. We found that Rb1 treatment can improve survival rate and body weight in Dox treated mice group. Rb1 can attenuate Dox induced cardiac dysfunction and myocardium hypertrophy and interstitial fibrosis. The oxidative stress increase and cardiomyocyte mitochondrion destruction were improved by Rb1 treatment. Mechanism study found that Rb1’s beneficial role in DIC is through suppressing of autophagy and Nrf2 induced ferroptosis. This study shown that Ginsenoside Rb1 can protect against DIC by regulating autophagy and ferroptosis.

Introduction

Doxorubicin (Dox) is one of the most commonly used antitumor drugs in clinic that can target for various cancers. However, the cardiotoxicity induced by Dox can cause irreversible cardiac injury and result in heart failure, which greatly limit its wide clinical usage[1]. Based on the time elapse since the first Dox administration and the first sign of toxicity, type of cardiotoxicity induced by Dox can be divided into acute or chronic type. Most studies are focusing on chronic cardiotoxicity, while the acute type is less investigated[2]. Numerous in vivo and in vitro studies have implicated that transcription alteration, mitochondrial damage, reactive oxygen species (ROS) overburden, calcium overload etc are involved in the pathogenesis of DIC[3–5]. Among those changes, oxidative stress generated from intracellular metabolism dysregulation is vital to induce heart damage[6, 7]. These molecular processes then initiate different types of cell damage or cell death procedures like inflammation, apoptosis or autophagy, which contribute to the development of DIC[8].

According to literatures, the most convincing mechanism involved in DIC are apoptosis and autophagy. However, evidences about autophagy inhibition or activation in DIC is controversial[9]. Autophagy is a precisely regulated process for cells to degrade unneeded organelles and proteins within the lysosomes to maintain cellular health and provide metabolic resources for reuse. The disturbance of autophagy is involved in a number of heart diseases like diabetic cardiomyopathy, coronary artery disease, inherited hypertrophic cardiomyopathy and heart failure [10, 11]. Autophagy flux activation or inhibition all have been reported in DIC. Some studies reported that activating autophagy can ameliorate DIC[12, 13] While other studies showed that inhibition of autophagy can attenuates DIC [14, 15]. Apart from autophagy, a new form of death, ferroptosis, was reported in Dox treated mice[16]. Inhibition of ferroptosis significantly reduce Dox-induced cardiac injury and heart failure[16]. These results from different studies reflected that the precise molecular pathway under DIC is still not fully understand.
Ginsenoside Rb1 is one of the major compounds of the widely used traditional Chinese herb Ginseng. The focus of Ginsenoside Rb1 are increasing since the discovery of its benefit on many systems like cardiovascular, central nervous and alimentary [17]. Studies have shown that Ginsenoside Rb1 protects acute ischemic myocardium through stimulation of AMPKa-mediated mitophagy and Ang II-induced abdominal aortic aneurysm via its anti-inflammatory effect[18]. Ginsenoside Rb1 exerts its anti-inflammatory, anti-apoptosis, autophagy and energy regulation effect in acute ischemic myocardium, Ang II-induced abdominal aortic aneurysm and even diabetic cardiomyopathy[18–20]. Despite the numerous benefits of Ginsenoside Rb1 on cardiovascular diseases, whether it can exert a protection role in DIC is unclear. In this study, we decide to explore the effect of Ginsenoside Rb1 on DIC through in vivo study and uncovering the potential mechanism of its function.

Methods and materials

Chemical and regents

Doxorubicin (S1208), Ginsenoside Rb1(S3924), were purchased from Selleck Chemicals (Houston, TX, USA).

Doxorubicin induced acute cardiotoxicity model:

Male C57BL/6 mice, 8–10 weeks old, were purchased from Beijing Huafukang Bioscience Co. Inc (Beijing, China). These mice were randomly divided into four treatment groups: saline group, Ginsenoside Rb1 group (Rb1), Doxorubicin treatment group (Dox), Doxorubicin + Ginsenoside Rb1 treatment group (Dox + Rb1). All mice were received free food and water with 12h light/ dark cycle in an environment with controlled temperature and humidity. After 1 week acclimatization of new environment, mice were used for experiment. To establish an acute DIC model, mice in Dox and Dox + Rb1 group received a single dose of 20mg/kg Dox via intraperitoneal injection. To observe the effect of Rb1 on DIC, mice in Rb1 and Dox + Rb1 group were given daily gavage of Rb1 (40mg/kg) 3 days before DOX injection and kept the same dose gavage daily till sacrificed. The saline group received equal volume saline gavage daily before harvest. All mice were weighted daily to record their body weight change. The experiment mice were taken for echocardiography study at the fifth day after Dox injection and sacrificed at day 7. The dose of Dox and Rb1 was referenced to previous articles[18, 21].

Transmission electron microscopy (TEM)

Mice heart samples, no more than 1 mm³, were quickly harvested and fixed in prepared fixative reagent (Servicebio, G1102, Wuhan, China) for TEM. Then samples were washed three times with 0.1M phosphate buffer (PH 7.4), each for 15min. The light-avoiding tissues were post fixed with 1% OSO4 in 0.1M phosphate buffer (PH 7.4) for 2h at room temperature and again washed with 0.1M phosphate buffer for three times, each for 15min. After that, the samples were dehydrate with gradient ethanol at room temperature. Next, the specimens were penetrated with resin, and embedded in acetone and EMBed 812 (SPI, USA). The embedded samples were moved into 65°C oven to polymerize over 48h. The resin blocks
were cut into 60-80nm thickness by the Ultra microtome (Leica, UC7), and the tissues were fished out onto the 150 meshes cuprum grids with formvar film. Then, the cuprum grids underwent double staining in 2% uranium acetane saturated alcohol solution and 2.6% lead citrate without CO₂, each for 8 min. After dried overnight at room temperature, the cuprum grids were observed under TEM (HITACHA, HT7800, Japan) and taken for images.

**Histopathological analysis**

Heart samples were collected and washed twice with PBS before fixed in commercial animal tissue fixing kit (G1101, Servicebio Technology Co., Ltd, Wuhan, China). Then followed by embedding and sectioning, and were finally stained with hematoxylin and eosin (HE) and Sirius red. The slices were imaged and scanned by Pannoramic MIDI (3DHISTECH, Hungary).

**Western blot**

Heart samples of different treatment groups were collected and protein were extracted using the Nuclear and Cytoplasmic Extraction Kit (CWBlO, CW0199). The protein concentration was determined by BCA assay. For Western blot analysis, a total amount of 30-50ug protein was loaded to 10–12% SDS-PAGE and then transferred to PVDF membranes. Then, the membranes were blocked with 5% nonfat milk that dissolved in TBST at room temperature for 2h. Primary antibodies were incubated with membranes at 4°C overnight. The primary antibodies used in this study were: Anti-SQSTM1 / p62((Abcam, ab109012), Anti-LC3A/B(Cell Signaling,12741), Anti-GAPDH (Cell Signaling, 2118), Anti-NRF2(Invitrogen, PA5-27882), Anti-Ferritin (Abcam, ab75973), Anti-Glutathione Peroxidase 4 (Abcam, ab125066). The membranes were incubated with second antibody for 1h at room temperature before washed with TBST. The second antibodies used include: IRDye® 680RD Goat (Polyclonal) Anti-Mouse IgG (H + L) Antibody ((LI-COR Biosciences, 926-68070), IRDye® 800CW Goat (Polyclonal) Anti-Rabbit IgG (H + L) Antibody (LI-COR Biosciences, 926-32211). Signal detection was carried out by LI-COR Odyssey CLx system. Signal analysis was performed by Image Studio Lite Ver 5.2 software.

**Echocardiography**

Echocardiology study of different groups mice was carried out on the fifth day after the initial injection of Dox. Briefly, mice were cleaned before measurement and then anesthetized with isoflurane for detection. Two dimensional M-mode echocardiography was performed with Vevo 2100 imaging system (Visual Sonics, Toronto, Canada). The measurements were performed by an independent investigator who are blinded to this study. Left ventricular internal diameter during systole (LVID, s), LVID during diastole (LVID, d) was measured from M-mode recording at the level of the mid-papillary muscle level. Left ventricular ejection fraction (LVEF) and fractional shortening (LVFS) was calculated by the Vevo LAB workstation software (version 1.6.0) automatically.

**Biochemical analysis**

The level of oxidate stress markers MDA, GSH, SOD, were measured by commercial kits using supernatant extracted from heart tissue. All the procedures were performed according to the
manufacturer's instruction (all from Nanjing Jiancheng Bioengineering Institute, China)

**RNA-Seq analysis**

Total RNA was extracted using the TRIzol reagent (Vazyme, Nanjing, China) according to the manufacturer's protocol. The purity and qualification of RNA were evaluated by NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). Then RNA integrity assessment was performed on Agilent 2100 Bioanalyzer (Agilent Technologies, USA). Libraries were constructed by VAHTS Universal V6 RNA-seq Library Prep Kit according to the manufacturer's instructions. The transcriptome sequencing and analysis were conducted by OE Biotech Co. Ltd. (Shanghai, China). The libraries were sequenced on Illumina Novaseq 6000 platform and 150 bp paired-end reads were generated. The clean reads were mapped to the reference genome using HISAT. FPKM of each gene was calculated and the read counts of each gene were obtained by HTSeq-count. PCA analysis were performed using R (v 3.2.0) to evaluate the biological duplication of samples. Differential expression analysis was performed using the DESeq2. Q value < 0.05 and fold change > 2 or fold change < 0.5 was set as the threshold for significantly differential expression gene (DEGs). Based on the hypergeometric distribution, GO, KEGG pathway, enrichment analysis of DEGs were performed to screen the significant enriched term using R (v 3.2.0), respectively. Gene Set Enrichment Analysis (GSEA) was performed using GSEA software.

**Statistical analysis**

All data were expressed as the mean ± standard deviation and analyzed by GraphPad Prism 8.0 software. Student's *t*-test were used to determine the difference between two groups. For multiple group difference analysis, we use one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The survival curve was performed by the Kaplan-Meier curves. *P* value <0.05 was considered as statistically significant.

**Results**

**Rb1 decreases mortality and increase body weight in acute Dox treated mice**

Schematic diagram of this study was shown in Fig. 1A. Briefly, different group mice received basic Saline or Rb1 treatment daily. Three days later, experiment group had a single dose of Dox injection (20mg/kg) and sacrificed at the seventh day after injection for heart sample collection. As shown in Fig. 1B, the additional treatment of Rb1in Dox + Rb1 group can significantly improve mice survival rate as compared to Dox treatment alone. Injection of single dose of Dox can reduce mice body weight gradually, but this trend was attenuated by Rb1 treatment (Fig. 1C). Mice survival rate of Rb1group has no difference compared to Saline group.
Rb1 improves cardiac function in DIC mice

On the sixth day after Dox injection, mice cardiac function was measured by echocardiology. As shown in Fig. 2, Dox injection can impair left ventricular function as the left ventricular ejection fraction (LVEF) and fractional shortening (LVFS) was significantly reduced and end-systole left ventricular internal dimensions (LVID, s) was increase in Dox group compared to saline group. But these changes were markedly reversed by Rb1 administration, which indicated that Rb1 cotreatment can protect against Dox induce cardiac function impairment in mice (Fig. 2A-B).

Rb1 attenuates Dox induced cardiomyocyte hypertrophy and interstitial fibrosis

To further confirm the cardiotoxicity of acute Dox treatment on cardiomyocyte, we performed histological observations by using HE and Sirius red staining. Results shown that Dox can induce cardiomyocyte hypertrophy, disarray and interstitial fibrosis (Fig. 3A-B). However, after Rb1 treatment, the above-mentioned pathological changes were markedly reduced, which reflected Rb1 can attenuates myocardium injury and fibrosis.

Rb1 attenuated DOX-induced oxidative stress and mitochondrion destruction in cardiac tissue

The oxidative stress is reported to be associate with cardiac toxicity induced by Dox. The change of oxidative stress can be reflected by oxidant markers. In this study we measured level of oxidant marker SOD, MDA, GSH in different group mice heart. As shown in Fig. 4, the level of SOD, GSH level was significantly decreased and MDA content was highly upregulated. These changes were attenuated in Dox + Rb1 group (Fig. 4A-C). Mitochondrion is the palace where oxidative reaction happens, so we use TEM to observe whether myocardium mitochondrion change each group. Under electron microscope, myocardium mitochondrion in Dox group were vesicular. The crest and cristae were dissolved or disappeared (Fig. 4D). However, the crest and cristae change in Dox + Rb1 group is less severe than that in Dox group. These results demonstrated that Rb1 exert a protection role in DIC partially through suppression of oxidative stress.

Rb1 improve Dox induced cardiomyocyte autophagy dysfunction

To explore whether autophagy is involved in the pathogenesis of DIC, we performed WB analysis. Results from WB show that autophagy marker P62 and LC3 expression level in Dox group are highly upregulated (Fig. 5). However, after Rb1 treatment, levels of P62 and LC3 in Dox treated heart are significantly reduced (Fig. 5A-C). These results reflected Rb1 can attenuate DIC by regulation of autophagy.
Rb1 attenuate Dox induced cardiotoxicity by activating AMPK-mTOR pathway.

To further explore the possible mechanism of Rb1’s protection role upon DIC, we performed RNA-Seq analysis by using RNA-extracted from mice myocardium. Heat map illustrate the most significantly regulated genes detected within Dox and Dox + Rb1 group (Fig. 6A). The KEGG pathway enrichment was used to detect possible pathway that is responsible for Rb1’s improvement on DIC. The top 20 differentially expressed genes were depicted in Fig. 6B. Among those with significantly changed pathway, AMPK signaling pathway was highly downregulated. We then confirmed this change by WB analysis (Fig. 6C-E). The WB result shown that Rb1 can reduce the upregulation of pAMPK and downregulation of pmTOR in Dox group, which further demonstrated that AMPK and mTOR pathway is activated by Rb1 administration in Dox group mice.

Rb1 attenuate Dox induced ferroptosis via Nrf2 signaling

Ferroptosis is a new form of cell death discovered recently [16]. It has been reported that ferroptosis is activated in Dox induced cardiotoxicity. Therefore, we made an attempt to test whether it is activated in our study. Results from WB (Fig. 7) shown that ferroptosis marker protein FTH1 and GPX4 was significantly decrease in Dox group. These changes were reversed in Dox + Rb1 group. The Nrf2 signaling is an anti-ferroptosis signa. Therefore, we detected the Nrf2 expression level in WB. The level of Nrf2 in Dox group is downregulated and treatment of Rb1 can increase the expression level of Nrf2, which demonstrated that Rb1 can attenuate Dox induced ferroptosis via Nrf2 signaling.

Discussion

Doxorubicin is one of the most commonly used anti-tumor drugs. However, its usage was greatly hampered by its cardiotoxicity. So, it’s urgently needed to explore new treatments to overcome this side effect. Many reagents were reported to have a cardiac protection role on DIC, but only one drug, dexrazoxane, has been approved for clinical use to reduce the side effect of Dox in real world practice[22]. Thus, it’s necessary to unveil the underline mechanism of DIC and explore new potential chemicals to attenuated DIC.

Ginsenoside Rb1 is the main active component of the traditional Chinese herb ginseng, which have been proved to exert beneficial effect on many cardiovascular diseases[20, 23, 24]. One study reported that Ginsenoside Rb1 can attenuated DOX-induced cardiomyocytes injury and apoptosis[25]. But its role on DIC is not clear. Therefore, we performed this in vivo study to explore whether Ginsenoside Rb1 can have a protection role on DIC and the mechanisms of its function.

Results from our acute DIC mice models shown that Ginsenoside Rb1 can significantly improve mice survival rate after Dox treatment. The body weight in Dox + Rb1 group mice was also increased compared to Dox treatment alone. Meanwhile, Rb1 treatment can improve mice cardiac function and attenuate cardiomyocyte enlargement, disarray and interstitial fibrosis as observed in Dox group. What’s more, Rb1
can reduce oxidative stress and cardiac mitochondrion destruction. These results demonstrated that Ginsenoside Rb1 plays a beneficial role in acute DIC mice model.

Rb1 have been reported to exert its cardioprotective effect in myocardial ischemia/reperfusion injury and heart failure via regulation of autophagy (24, 25). Therefore, we wonder whether Rb1 can attenuate DIC through regulation of autophagy. In our study, autophagy marker of P62 and LC3 are highly upregulated in Dox treated mice. But both of the two proteins were downregulated after Rb1 treatment. However, in one rat DIC model, autophagy was suppressed as LC3 level is relatively lower than the Dox group[26]. The discrepancies among different studies maybe a result of the DIC model difference as there are great variation in Dox dosage, injection interval, observation time and even species difference in each DIC model[27]. During the observation period, we noticed that food intake of mice in Dox group was dramatically reduced after the injection of Dox, which may represent a state of starvation. In normal condition, starvation can induce autophagy process enhancement. Therefore, the increasing of P62 and LC3 may be partially caused by the Dox induced starvation.

To explore the mechanisms of how Ginsenoside Rb1 exert its protective role on DIC, we performed RNA-seq analysis. KEGG pathway enrichment shows that AMPK pathway is down regulated in DOX + Rb group as compared with DOX group. Some studies reported that Dox trigger autophagy in mice heart by activating AMPK, while other reports showed AMPK has no change or even inhibition (19–21). Normally, phosphorylated AMPK activate ULK and deactivate the inhibition effect of mTOR to initiated the process of autophagy. The first stage of autophagy is represented by autophagosome formation which is corelated with biomarker protein P62 and LC3 increase. Next, the mature autophagosome fuse with lysosome to form autolysosome, where cargos from autophagosomes are degraded by lysosome and protease. Therefore, the observed LC3 and P62 increase in Dox group is likely due to the excess activation of autophagosome formation (22, 23).

The AMPK-mTOR axis is a canonical pathway involved in energy sensation and autophagy regulation[28]. Other studies also reported this pathway dysregulation in DIC animal models. For example, Li et al reported dihydromyricetin protect against DIC through AMPK/mTOR activation[26]. In Liu’s study, the beneficial role of spinacetin on DIC is also through regulation of AMPK/mTOR pathway[29]. AMPK is a key molecular in regulating cellular energy homeostasis[30]. Activation of AMPK can target numerous downstream targets that involved in metabolic processes and autophagy. Under energy stress, the ratio of ATP/AMP decrease, which then activate AMPK signal. The AMPK pathway is highly connected with autophagy process. This indicated that the improvement of Rb1 on autophagy dysfunction in DIC mice is through inhibiting of AMPK pathway.

Ferroptosis is a novel form of cell death process which have been reported to be involved in DIC[16]. In our study we performed experiments to test whether ferroptosis exist in our DIC mice model. Results shown that several major markers of ferroptosis like FTH1, GPX4 were significantly downregulated after Dox treatment which strongly indicated ferroptosis activation. However, these changes were reversed after Rb1 cotreatment. The oxidative stress burden induced by Dox can result in the imbalance of
antioxidative system in vivo, as reflected by the increase of SOD and GSH content. The overproduction of ROS interacted with iron and initiated the damage of lipid membrane, which induced the activation of ferroptosis. Nrf2 is a canonical antioxidative protein. When oxidative stress is high, the Nrf2 translocate to the nuclear to bind ARE, which further result in the gene expression upregulation of detoxification and antioxidant enzymes[31]. In our study, the additional treatment of Rb1 in Dox group can induce Nrf2 expressing increase. The protein level of FTH1, GPX4 was also increased after Rb1 treatment. This observation strongly support that Rb1 can protect Dox induced cardiac damage via Nrf2 regulated ferroptosis. Similar mechanism was also reported in another Dox treated mice model as well as in Arsenic trioxide induced cardiotoxicity, which is also an anti-tumor drug[32, 33].

Collectively, our work demonstrates that Ginsenoside Rb1 can alleviated DIC (Fig. 8). The potential mechanism of its beneficial role is mediated by autophagy and ferroptosis suppression, which are via AMPK and Nrf2 signal pathway separately. The data in our study suggests that Ginsenoside Rb1 is a potential agent to reduce acute DIC. Further in vitro study about its protection role in DIC are needed.

Conclusion

Our study provides evidence that Ginsenoside Rb1 can exert a protection role in dox induced cardiotoxicity. We demonstrated that the Ginsenoside Rb1 attenuate dox induced cardiotoxicity via regulation of autophagy and ferroptosis. The use of Ginsenoside Rb1 may be a novel choice for dox induced cardiotoxicity improvement.

Declarations

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Competing Interests The authors declared no conflict of interest to disclose.

Author Contributions Xiaoyan Zhao and Jianzeng Dong designed the study. Yafei Zhai, Jinmeng Bai, and Ying Peng conducted the experiment and Yafei Zhai drafted the manuscript. Jinhua Cao, Guangming Fang, Yiming Dong, Ze Wang collected and analyzed the data. Yanyu Lu, Mengyu Wang and Mengduan Liu provided technical support for echocardiology. Yangyang Liu and Xiaowei Li revised the manuscript. All authors read and approved the final manuscript.

Data Availability Statement All data generated or analyzed during this study are available from the corresponding author on reasonable request.

Ethics approval All animal experiments were performed according to the guidelines of the Ethics Committee for Laboratory Animals of Zhengzhou University and followed national and institutional guidelines.
References


Figures
Figure 1

Ginsenoside Rb1 (Rb1) improve survival rate and body weight in doxorubicin (Dox) treated mice. A, schematic diagram of experiment design; B, survival curve of different group mice; C, Body weight change in each group mice were recorded during the observation period.
Figure 2

Rb1 improve Dox induce cardiac dysfunction in mice. A. Representative image of M-mode echocardiography in different group mice; B, Statistical analysis of left ventricular ejection fraction (LVEF), fractional shortening (LVFS) and left ventricular internal diameter end systole (LVIDs), (n=5 per group). *P 0.05 Dox vs Saline group, **P 0.01 Dox vs Saline group, # P 0.05 Dox+Rb1 vs Dox group, ## P 0.05 Dox+Rb1 vs Dox group
Figure 3

Rb1 alleviates Dox induced cardiac injury and interstitial fibrosis.

A, cardiomyocytes were evaluated by HE staining; B, Representative image of Sirius red staining
Figure 4

Rb1 alleviates Dox induced cardiac oxidative stress and mitochondrion destruction.

A, Myocardium SOD activity; B, Myocardium MDA content. C, Myocardium GSH activity. n=5 *P 0.05 Dox vs Saline group.** P 0.01 Dox vs Saline group,# P 0.05 Dox+Rb1 vs Dox group, ## P 0.01 Dox+Rb1 vs Dox group. D, Representative myocardium mitochondrion image under TEM. Red arrow indicated mitochondrion crest and cristae dissolve or disappear.
Figure 5

Rb1 suppress autophagy in Dox treated mice myocardium.

A, Representative western blot image of autophagy marker LC3 and P62 (n=3 per group); B&C, quantitative data of LC3- and P62 protein expression. * $P<0.05$ Dox vs Saline group, ** $P<0.01$ Dox vs Saline group, # $P<0.05$ Dox+Rb1 vs Dox group, ## $P<0.01$ Dox+Rb1 vs Dox group
Figure 6

Rb1 attenuate Dox induced cardiotoxicity by down regulating AMPK signaling.

A. Representative heatmap of gene expression in Dox and Dox+Rb1 group (n=3 per group); B, KEGG pathway enrichment of different expression genes between Dox and Dox+Rb1 group; C, D and E, Representative western blot image and quantitative measurement of pAMPK and pmTOR protein expression level. * P 0.05 Dox vs Saline group, ** P 0.01 Dox vs Saline group, # P 0.05 Dox+Rb1 vs Dox group, ## P 0.01 Dox+Rb1 vs Dox group
Figure 7

Rb1 inhibits Dox induced ferroptosis by down regulating Nrf2 signaling.

A. Representative western blot result of FTH1, GPX4 and Nrf2; B, C and D, quantitative measurement of FTH1, GPX4 and Nrf2 expression. * $P$ 0.05 Dox vs Saline group, ** $P$ 0.01 Dox vs Saline group, # $P$ 0.05 Dox+Rb1 vs Dox group, ## $P$ 0.01 Dox+Rb1 vs Dox group
Figure 8

Schematic diagram summarizing the potential signaling pathways referred to the protective effect of Ginsenoside Rb1 on acute dox induced cardiotoxicity.