

Gastrodin prevents myocardial injury in sleep-deprived mice by suppressing ferroptosis through SIRT6

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Abstract

Gastrodin (GAS), a bioactive compound derived from the orchid plant *Gastrodia elata*, exhibits numerous pharmacological effects. However, its effect on sleep deprivation (SD)-induced cardiac injury and the mechanisms are unknown. This study established SD mice model using a modified multiple platform water method and induced ferroptosis model in H9C2 cells using Erastin. The heart rate of mice was measured, and myocardial and mitochondrial structures were visualized using hematoxylin and eosin (H&E) staining and transmission electron microscopy (TEM). Myocardial injury, oxidative stress indicators and, Fe^{2+} levels were detected by the kit method. The reactive oxygen species (ROS) levels were detected by immunofluorescence, and SIRT6 and ferroptosis-associated protein expression levels were detected by Western blot. Reduced heart rate and abnormalities in myocardial tissue and mitochondrial structure were ameliorated in the SD group of mice after GAS treatment. GAS treatment reduced ROS levels in Erastin-induced H9c2 cells. GAS treatment reduced atrial natriuretic peptide (ANP), creatine kinase (CK), lactate dehydrogenase (LDH), malondialdehyde (MAD), and Fe^{2+} levels, and increased superoxide dismutase (SOD) and glutathione (GSH) levels in the SD and Erastin groups. Western blot showed that GAS treatment increased the expression of sirtuin 6 (SIRT6), solute carrier family 7 member 11 (SLC7A11), and glutathione peroxidase 4 (GPX4) and decreased the expression of P53 in SD and Erastin groups. The SIRT6 inhibitor OSS_128167 (OSS) reversed GAS treatment of Erastin-induced ferroptosis in H9C2 cells. These observations propose that GAS prevents myocardial injury in sleep-deprived mice by suppressing ferroptosis through SIRT6.

1. Introduction

Sleep is an indispensable physiological process, playing a critical role in the maintenance of bodily health equilibrium (Stich et al., 2022). Sleep quality is considered a key factor in the occurrence of cardiovascular diseases (Bishop et al., 2023). In contemporary society, people are facing increased social pressure and resulting sleep deprivation. As a stressful stimulus, SD causes disturbances in physiological function and metabolic processes. Specifically, SD leads to an elevation in metabolic rates, concomitantly with an increase in ROS, attenuates the robustness of the immune defense mechanism, and culminates in the diminution of the body's antioxidative capabilities (Pandey and Kar, 2018). The phenomenon of SD is intricately associated with a gamut of cardiovascular ailments, encompassing myocardial ischemia, hypertension, and cardiomyopathy. It induces pathophysiological alterations, including myocardial infarction, myocardial ischemia, and myocardial fibrosis (Chen et al., 2022). Additionally, SD incites the synthesis of pro-inflammatory cytokines and disrupts the secretion of critical hormones, namely melatonin, cortisol, and angiotensin (Thompson et al., 2022). Therefore, it is essential to prevent and treat SD-induced myocardial injury and to reveal its underlying mechanisms.

Ferroptosis is defined as a programmed cell death brought on by aberrant iron metabolism as a result of excessive lipid metabolism (Yan et al., 2021). Cardiomyocytes are now considered non-regenerative cells in the body, and it is generally accepted that the more functionally normal cardiomyocytes there are in cardiovascular diseases, the higher the cellular vitality and the better the cardiac function (Yao et al.,

2024). Iron-dependent lipid peroxidation accumulation, which causes cellular damage, is one of the pathogenic mechanisms of various cardiac diseases, including myocardial infarction and myocarditis (Zhang and Guo, 2023). Increasing evidence supports that SD precipitates myocardial tissue damage through the induction of ferroptosis (Yuan et al., 2024). Research indicates that ferroptosis can be involved in a variety of cardiomyocyte-injurious diseases by disrupting iron-related metabolic homeostasis, oxidative and antioxidant systems, and lipid metabolism, and that protection of cardiomyocytes can be achieved by inhibiting ferroptosis and its associated pathways (Li et al., 2021). The ferroptosis inhibitor Ferrostatin-1 (Fer-1) has been shown to alleviate myocardial damage induced by ischemia-reperfusion injury (Luo et al., 2022). Previous study showed that SD induces ferroptosis in mice myocardium, emphasizing the importance of studying myocardial damage through ferroptosis inhibition, a topic gaining increasing scholarly focus (Cao et al., 2023a).

Gastrodia elata, an herb esteemed within traditional Chinese medicine, flourishes in the mountainous terrains of East Asia, predominantly originating from the Yunnan and Guizhou regions of China. The bioactive component GAS is extracted from the tubers of *Gastrodia elata*. Research has elucidated that GAS exhibits a broad spectrum of pharmacological properties and manifests therapeutic efficacy in a variety of cardiovascular diseases (Xiao et al., 2023). GAS attenuates cellular senescence in vascular endothelium by regulating NRF2 and OH-1 expression, subsequently reducing the prevalence of stroke events (Tong et al., 2024). Furthermore, when combined with aspirin, GAS has been identified to inhibit cardiomyocyte pyroptosis, thus addressing myocardial dysfunction (Dong et al., 2022). GAS also safeguards against homocysteine-induced venous endothelial cell injury by upregulating PI3K, p-Akt, and eNOS expressions (Chen et al., 2021). Beyond these effects, GAS has been found to counteract cardiac ailments such as myocardial hypertrophy, hypertension through its antagonistic actions on oxidative stress, inflammation, apoptosis, autophagy, and lipid dysregulation (Chen et al., 2021, Wang et al., 2024).

The study implemented an adjusted modified multi-platform method to induce SD model and utilized Erastin to induce a ferroptosis model in H9c2 cells. The study delved into potential molecular mechanisms by which GAS ameliorated myocardial injury and ferroptosis induced by SD.

2. Materials and methods

2.1. Chemicals and reagents

Gastrodin (with a purity exceeding 98%, 62499-27-8) was acquired from Dalian Meilun Biotechnology Co., Ltd. Additionally, Erastin (HY-15763), Fer-1 (HY-100579), SIRT6 (HY-P80322), and OSS (HY-107454) were procured from the Master of Small Molecules (MCE, Shanghai, China). The following assay kits were sourced from Solarbio (Beijing, China): SOD activity (BC1075), CK activity (BC1145), GSH content (BC1175), MDA content (BC0025) and Ferrous ion content detection kit (BC5415). The LDH assay kit (A020-2) was acquired from Njjcbio (Nanjing, China). ANP ELISA kit (JL20612) was acquired from Jianglai biology (Shanghai, China). ROS Kit (S0033S) was acquired from Beyotime (Shanghai, China).

Antibodies for β -actin (AC026), P53 (A19585), SLC7A11 (A2413), and GPX4 (A11243) were sourced from Abclonal (Wuhan, China).

2.2 Animals and treatment

Sixty male C57BL/6 mice (average weight: 22 ± 2 g, aged between 8–10 weeks) were obtained from Hangzhou Ziyuan Laboratory Animal Technology Co., Ltd. The animals were subsequently segregated into six groups: Control group, SD group, GAS groups (6.25, 12.5, and 25 mg/kg), and Fer-1 group (1 mg/kg). In the study, the control group of mice was administered distilled water via oral gavage for nine consecutive days. The GAS groups received gastrodin at varying concentrations through the same route for a duration of 9 d, while the Fer-1 group was treated with intraperitoneal injections of Fer-1 over the same period. Following this, mice were subjected to SD for 48 h using a modified multi-platform method, with the exclusion of the control group. This method allowed the mice to freely navigate the platforms for drinking and feeding purposes. Upon exhaustion, decreased muscle tone caused the mice to fall into the water, awakening them and thus achieving SD. Following sleep deprivation, heart rates of the mice were recorded, after which the animals were sacrificed and serum as well as heart tissue were harvested.

2.3 Hematoxylin & Eosin Staining

Myocardial tissues were preserved in 4% paraformaldehyde for a duration of 48 hours subsequent to collection. Subsequently, these tissues underwent treatment with xylene and a series of ethanol dilutions. The specimens were then embedded, sectioned, and subjected to deparaffinization. Following this, they were stained with Hematoxylin and Eosin, thoroughly rinsed, dehydrated in a graded ethanol series, decolorized with xylene, and finally prepared for microscopic examination by being washed and mounted (Cao et al., 2023a).

2.4 Assessment of ANP, CK, LDH, MDA, GSH, SOD and Fe^{2+} content

Assessment of ANP, CK, LDH SOD, GSH, and MDA levels in serum and Fe^{2+} level in myocardial tissues of mice. Additionally, H9c2 cells were collected and assayed for ANP, CK, LDH, SOD, GSH, MDA, and Fe^{2+} levels. All of the above indicators were detected through the use of specific assay kits.

2.5 Transmission electron microscope

After being collected, the myocardial tissue samples were stored in 2.5% glutaraldehyde for 24 h. These tissues were subsequently dehydrated through a series of ethanol concentrations after osmium acid treatment. Embedding was performed after transparency using xylene and cut into ultrathin sections. These sections were then stained with a 2% solution of uranyl acetate and lead citrate, thoroughly rinsed, and prepared for observation under an electron microscope.

2.6 Cell Culture and Grouping

H9c2 cells were cultured in sterile culture flasks in high glucose medium with 10% fetal bovine serum at 37°C in an environment of 5% CO₂. Subsequently, H9c2 cells were grouped and treated according to the experimental requirements. The divisions included control group, Erastin 10 µM group, Erastin + GAS (6.25 µM, 12.5 µM, and 25 µM) groups, Erastin + Fer-1 10 µM group, Erastin + OSS 50 µM group, and Erastin + GAS 25 µM + OSS 50 µM group. Cells in all groups except the control group were pre-induced with Erastin 10 µM for 4 h, followed by drug administration treatment.

2.7 ROS detection

To detect the level of ROS in H9C2 cells, a fluorescent probe, DCFH-DA, diluted at 1:1000 was utilized. Except for the control group, each group was pretreated with 10 µM Erastin for 4 h and then incubated with the corresponding drug-containing medium for 24 h. H9C2 cells were resuspended by centrifugation, then the fluorescent probe was added and incubated at 37°C for 20 min, protected from light. 1200 rpm/min centrifugation was performed, the dilution was discarded, and the cells were rinsed with PBS to eliminate fluorescent probe residues that had not entered the cells. The fluorescence intensity of ROS was detected under a fluorescence microscope (488 nm).

2.8 Western blot assay

Myocardial tissue and H9c2 cells proteins were fragmented and added to RIPA lysis buffer and incubated at 0°C for 30 min. Protein quantification was then performed using the BCA kit to calculate concentrations and prepare protein samples. Following quantification, the samples were subjected to electrophoresis on a 12% SDS-PAGE gel. Proteins were then transferred to methanol-activated PVDF membranes, which were closed with 5% skimmed milk for 2 h and washed three times with TBST solution for 20 min each time. Overnight incubation at 4°C with the target primary antibody (1:1000) preceded further washes with TBST solution and a 1 h incubation with the secondary antibody (1:5000). ELC luminescence kits were used to view the proteins and documented through photography for recording.

2.9 Statistical analysis

Data analysis was conducted using GraphPad Prism 9.0, with results presented as mean ± SD. A one-way analysis of variance (ANOVA) was employed to assess the differences among the groups, with a *p*-value of less than 0.05 considered to be statistically significant.

3. Results

3.1 GAS ameliorated SD-induced cardiac injury in mice.

A Modified multi-platform method was utilized to induce SD mice. Compared to the control group, myocardial tissues in the SD group exhibited pronounced nuclear condensation, disrupted myocardial tissue texture, and disorganized or fragmented myofibrillar architecture (Fig. 1a). Treatment with GAS and Fer-1 reduced myocardial tissue damage areas significantly, with the most pronounced effect

observed in the GAS 25 mg/kg group. Figure 1b shows that the heart rate in SD-induced mice significantly increased compared to the control group but decreased post-GAS treatment, particularly at a dosage of 25 mg/kg. Compared with the normal group, the SD group showed increased levels of ANP, CK, LDH, MDA, and Fe²⁺ as well as decreased levels of SOD and GSH, which were significantly inhibited by GAS and Fer-1 treatment in a dose-dependent manner. The study demonstrated that GAS alleviated SD-induced cardiac injury in mice.

3.2 GAS inhibited SD-induced ferroptosis in mice

Compared to control mitochondria, myocardial tissues in the SD group highlighted mitochondrial anomalies such as edema, reduced cristae density, membrane ruptures, and ridge effacement or disappearance the in SD group (Fig. 2a). These mitochondrial damages were effectively inhibited by GAS and Fer-1 treatments, with GAS 25 mg/kg nearly restoring mitochondrial integrity to the control group levels. The above results indicate that GAS significantly inhibited SD-induced morphological changes in myocardial mitochondria.

As shown in Fig. 2b-f, SD induced a decline in SIRT6 protein levels, which GAS treatment reverted to the control group. Analysis of ferroptosis-related proteins disclosed an upsurge in P53 and a downturn in SLC7A11 and GPX4 expressions compared to the control group, alterations that were reversed post-GAS and Fer-1 treatments, most notably in the GAS 25 mg/kg group.

3.3 GAS inhibited Erastin-induced ferroptosis in H9c2 cells.

As shown in Fig. 3a-g, myocardial injury, oxidative stress, and Fe²⁺ metrics were measured in Erastin-induced H9c2 cells. The contents of ANP, CK, LDH, MDA, and Fe²⁺ were increased, and the contents of SOD and GSH were decreased in Erastin-induced H9c2 cells. After treatment with GAS and Fer-1, the above parameters were restored, especially in the GAS 25 µM group. Meanwhile, ROS levels were examined in H9c2 cells. The fluorescence intensity of ROS was significantly enhanced in the Erastin group compared with the control group; the fluorescence intensity was decreased in the GAS 12.5, 25 µM, and Fer-1 groups compared to the Erastin group, and the decrease in the fluorescence intensity was especially obvious in the 25 µM group (Fig. 3h-i).

3.4 GAS reversed Erastin-induced aberrant expression of SIRT6 and ferroptosis-related proteins in H9c2 cells

The study conducted an evaluation of SIRT6 and ferroptosis-related protein expressions within an Erastin-induced ferroptosis model in H9c2 cells (Fig. 4). The results of the study showed that elatin pretreatment resulted in a decrease in the expression of SIRT6, SLC7A11, and GPX4, along with an increase in the expression of P53 in H9c2 cells compared to control proteins. GAS treatments led to an increase in the expressions of SIRT6, SLC7A11, and GPX4, while the expression of P53 was decreased, and the concentration of 25 µM showed the most obvious and significant dose-dependent recovery effect.

3.5 Gastrodin inhibited Erastin-induced ferroptosis in H9c2 cells through the upregulation of SIRT6

The SIRT6 inhibitor OSS was added to study the relationship between SIRT6 and ferroptosis-related proteins in H9c2 cells (Fig. 5). Compared to the control group proteins, Erastin-induced H9c2 cells demonstrated a decreased expression of SIRT6, SLC7A11, and GPX4 while increasing the expression of P53. The GAS groups effectively inhibited the reduction of SIRT6, SLC7A11, GPX4 expression and the increase of P53 expression compared to the Erastin proteins. However, co-treatment with GAS and SIRT6 inhibitor OSS suppresses the therapeutic effect of GAS, leading to reduced expression of SIRT6, SLC7A11, and GPX4, and increased expression of P53.

4. Discussion

Research indicates that SD elevates markers of myocardial injury and oxidative stress levels, thus becoming a risk factor for cardiovascular diseases (Jówko et al., 2018). SD also has a significant impact on the emergence of hypertension and morphological changes in the myocardium (Münzel et al., 2021). Contemporary epidemiological inquiries have shown that SD is strongly associated with the incidence of cardiovascular disease, accentuating the imperative for pharmacological interventions targeting SD-induced myocardial impairment. GAS can effectively inhibit various cardiovascular diseases, including myocardial ischemia/reperfusion injuries (Zhu et al., 2018). However, it is yet unclear how GAS contributes to myocardial damage brought on by SD. The study observed an aberrant rise in heart rates among the SD group. Meanwhile, the H&E staining results in the SD group also showed disturbed arrangement of cardiomyocytes in myocardial tissues, uneven cytoplasmic coloring, myocardial fibers were seen to be broken, and the nuclei of cardiomyocytes in the damaged area were solidified. After GAS treatment, the heart rate of the mice decreased and returned to normal. Correspondingly, myocardial injury in mice was improved after GAS treatment, especially in the GAS 25 mg/kg group, where the cardiomyocytes were neatly aligned, with clear boundaries, uniformly colored cytoplasm, and centered nuclei. The myocardial fibers were regular, and no fracture was observed.

LDH, an indicator of myocardial injury, abounds in myocardial tissues and is liberated into the bloodstream consequent to myocardial damage (Xia and Sun, 2023). CK is also a marker of myocardial infarction and its total activity is commonly used to assess the extent of myocardial injury (Zhou et al., 2024). SOD operates as an antioxidative enzyme, fortifying cells against oxidative harm. Increased MDA levels signify cellular membrane compromise resulting from oxidative stress, closely linked to cardiovascular disease progression (Amin et al., 2018). GSH is an intrinsic antioxidant that reduces oxidative stress damage during ferroptosis (Xia et al., 2024). The absorption, transportation, storage, and utilization of ferric ions all affect the susceptibility to ferroptosis. Extracellular Fe^{3+} enters the intracellular and is converted to Fe^{2+} . Excessive intracellular Fe^{2+} will react to produce hydroxyl radicals thereby damaging the cell membrane and leading to the onset of cellular ferroptosis (Dixon and Olzmann, 2024). To substantiate the myocardial impairment engendered by SD, we assessed myocardial

injury, oxidative stress markers, and Fe^{2+} levels. discovering anomalously heightened concentrations of CK, LDH, MDA, and Fe^{2+} in sleep deprivation mice, alongside markedly diminished SOD and GSH levels. Treatment with GAS significantly reversed the abnormal changes in the above molecular markers and attenuated the level of oxidative stress in myocardial injury. Additionally, the study demonstrated the ability of GAS to attenuate sleep deprivation-induced myocardial injury in mice. Similarly, we evaluated the aforementioned markers and Fe^{2+} levels in an Erastin-induced ferroptosis model of H9c2 cells, finding increased CK, LD, MDA, and Fe^{2+} levels with reduced SOD and GSH levels in Erastin-treated H9c2 cells, while GAS rectified these deviations. Thus, our findings confirm the efficacy of GAS in ameliorating myocardial tissue and Erastin-induced H9c2 cell injury.

The morphological characteristics of ferroptosis are primarily manifested by ultrastructural changes in mitochondria (Otasevic et al., 2021). Ferroptosis is primarily characterized by the Fenton reaction caused by increased Fe^{2+} and the accumulation of ROS damage due to reduced GPX4 (Henning et al., 2022). Ferroptosis leads to mitochondrial damage and an increase in ROS in H9c2 cells, whereas GAS ameliorates mitochondrial morphological damage and reduces ROS accumulation in H9c2 cells. P53, serving as a pivotal transcription factor, plays an instrumental role in the pathogenesis and therapeutic approaches of cardiac disorders, mediating through anti-angiogenic activities, apoptotic induction, autophagy modulation, and metabolic regulation (Wang et al., 2023). Elevated levels of P53 occur in heart failure, myocardial injury, and a variety of other diseases (Evans and Mann, 2013, Su et al., 2021). It has been observed that myocardial infarction and cardiomyopathy are linked to reduced GPX4 expression, with the in vitro inhibition of GPX4 leading to ferroptosis. SIRT6 deficiency in mice leads to the occurrence of myocardial fibrosis, accompanied by an increase in P53 expression (Zhang et al., 2022). It has been shown that P53 mediates the suppression of SLC7A11 expression, thus inhibiting cystine transport, which is fundamental for GSH biosynthesis (Zeng et al., 2022). Cystine is a raw material for the synthesis of GSH, and a decrease in SLC7A11 leads to a block in cystine uptake indirectly leading to a decrease in GPX4 (Zhang et al., 2024).

SIRT6 emerges as a quintessential member of the Sirtuin family, endowed with capabilities to attenuate oxidative stress, counter cellular autophagy, avert apoptosis, and forestall cellular aging and tumorigenesis (Liu et al., 2021). Increasing studies have revealed the connection between SIRT6 and cardiac protection, highlighting its pivotal role in cardiomyocyte preservation (Liu et al., 2024). The deficiency of SIRT6 in mice results in myocardial mitochondrial damage akin to that observed in aged mice (Divya et al., 2024). Moreover, the overexpression of SIRT6 mitigates oxidative stress-induced damage and cardiotoxicity in myocardial cells induced by doxorubicin (DOX) (Pillai et al., 2021). Deficiency of SIRT6 leads to cardiomyocyte injury and mitochondrial dysfunction, whereas its overexpression attenuates various cardiovascular diseases, suggesting that it plays an indispensable role in cardiomyocyte viability (Divya et al., 2024). A particular study showed that inhibiting the expression of SIRT6 protein exacerbates iron death mediated by P53 in acute lung injury in mice, while inhibition of P53 attenuates the exacerbation of iron death injury by inhibiting SIRT6 expression (Cao et al., 2023b). To explore the relationship between sirt6 and ferroptosis, we measured the expression of

ferroptosis-related proteins. Western blot assays showed a decline in SIRT6, SLC7A11 and GPX4 protein levels, concurrently with an elevation in P53 expression. GAS exerted an influence on the aberrations induced by SD in SIRT6, SLC7A11, and GPX4 protein expressions within the mice myocardium, enhancing SIRT6, SLC7A11, and GPX4 levels but reducing P53 expressions. The same expression was observed in Erastin-induced H9c2 cells, where Erastin-induced a significant downregulation of SIRT6, SLC7A11, GPX4, and an upregulation of P53. Remarkably, GAS reversed Erastin induced abnormal changes in the above proteins in H9c2 cells.

To further explore the relationship between SIRT6, P53, and ferroptosis, as well as the influence of GAS on them. This study added the SIRT6 inhibitor, OSS, to Erastin-induced H9c2 cells to block SIRT6 activity. It was found that the therapeutic effect of GAS on Erastin-induced H9c2 cells was inhibited by the addition of OSS, and the expression of SIRT6, P53, SLC7A11, and GPX4 were restored to the level of the Erastin group. The above results suggest that GAS may regulate the expressions of P53, SLC7A11, and GPX4 by activating SIRT6 and thus inhibiting ferroptosis in cardiomyocytes.

5. Conclusion

As shown in Fig. 6, GAS exerts a protective role against SD-induced myocardial injury. GAS reduced the levels of ANP, CK, LDH, MDA and Fe^{2+} both in vivo and in vitro, and both increased the levels of SOD and GSH. GAS reduced SD-induced mitochondrial morphological damage in mice and Erastin-induced ROS accumulation in H9c2 cells. GAS upregulated the expression of SIRT6 and the ferroptosis-related protein SLC7A11 and GPX4, along with a reduction in P53 expression. Thus, the expression alterations of these proteins could be inhibited by OSS. Consequently, the study posits that Gastrodin prevents myocardial injury in sleep-deprived mice by suppressing ferroptosis through SIRT6.

Declarations

Authors contributions

Yin Cao and Zipeng Gong designed the experiment. Yi Wu drafted the paper. Yi Wu and Yuping Miao completed the experiment. Yi Wu and Yuping Miao analyzed the data. Zipeng Gong revised the paper. Yin Cao and Zipeng Gong involved in conceptualizing, analyzing, and interpreting the paper, critically revising the manuscript, giving final approval, and agreeing to take responsibility for all aspects of the work to ensure completeness and accuracy. The authors confirm that all data were produced internally and affirm that no paper mill was involved.

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Data availability

The corresponding author can provide the data that supported the study's findings upon request.

Competing interests

No conflicts of interest are disclosed by the authors.

Ethical approval

Animal Ethics of Anhui University of Traditional Chinese Medicine (Approval Number: AHUCM-mouse-2020066) approved all the animal experiment in this study.

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Figures

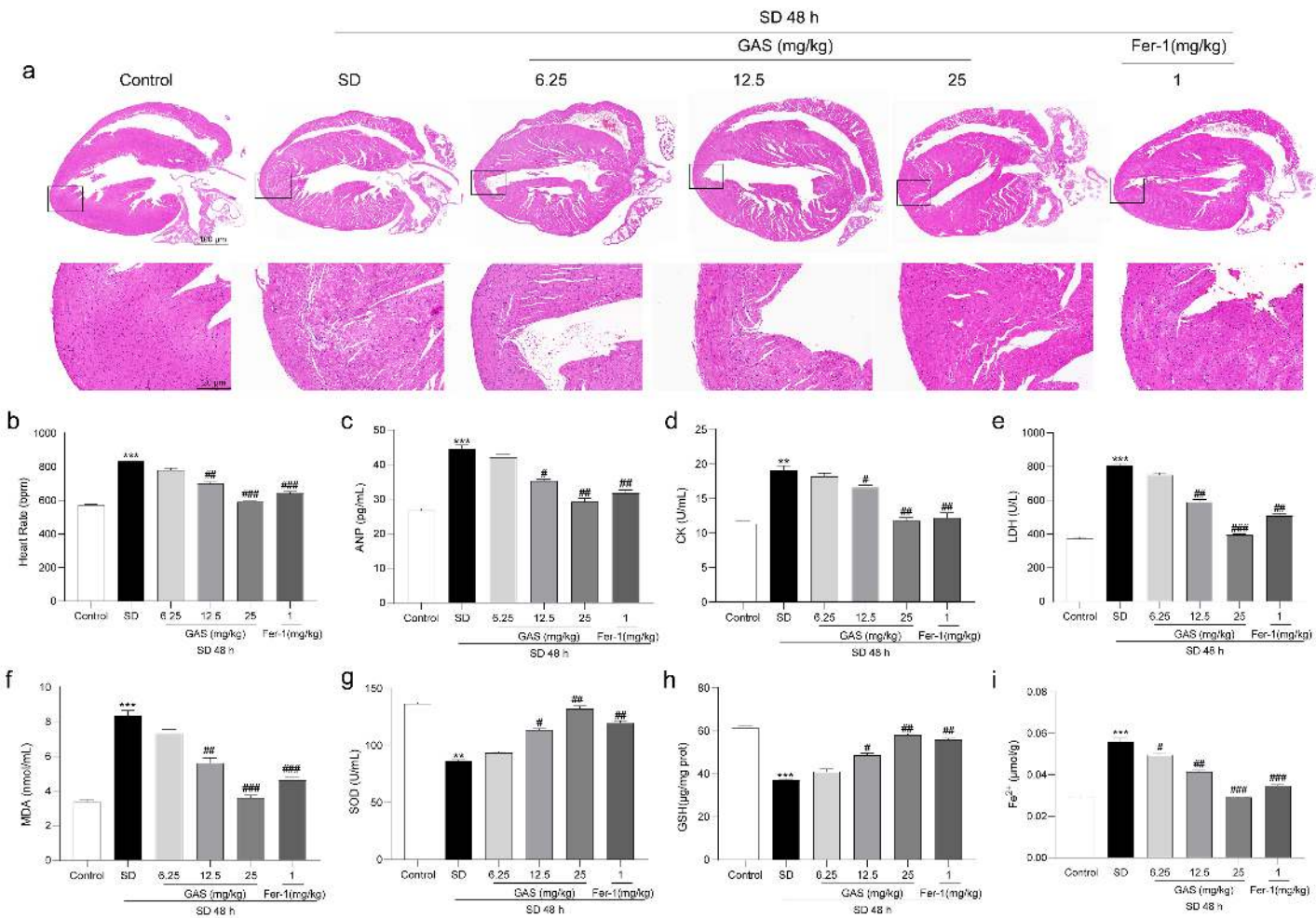


Figure 1

GAS ameliorated SD-induced cardiac injury in mice. H&E staining n = 3 / group. Scale bar: 100 μ m or 20 μ m (a). GAS inhibited the abnormal changes of heart rate (b), ANP (c), CK (d), LDH (e), MDA (f), SOD (g), GSH (h) and Fe^{2+} (i) in SD mice. n = 5 / group. * P < 0.05, ** P < 0.01, *** P < 0.001, contrast to control group. # P < 0.05, ## P < 0.01, ### P < 0.001, contrast to the SD group. n = 5 / group.

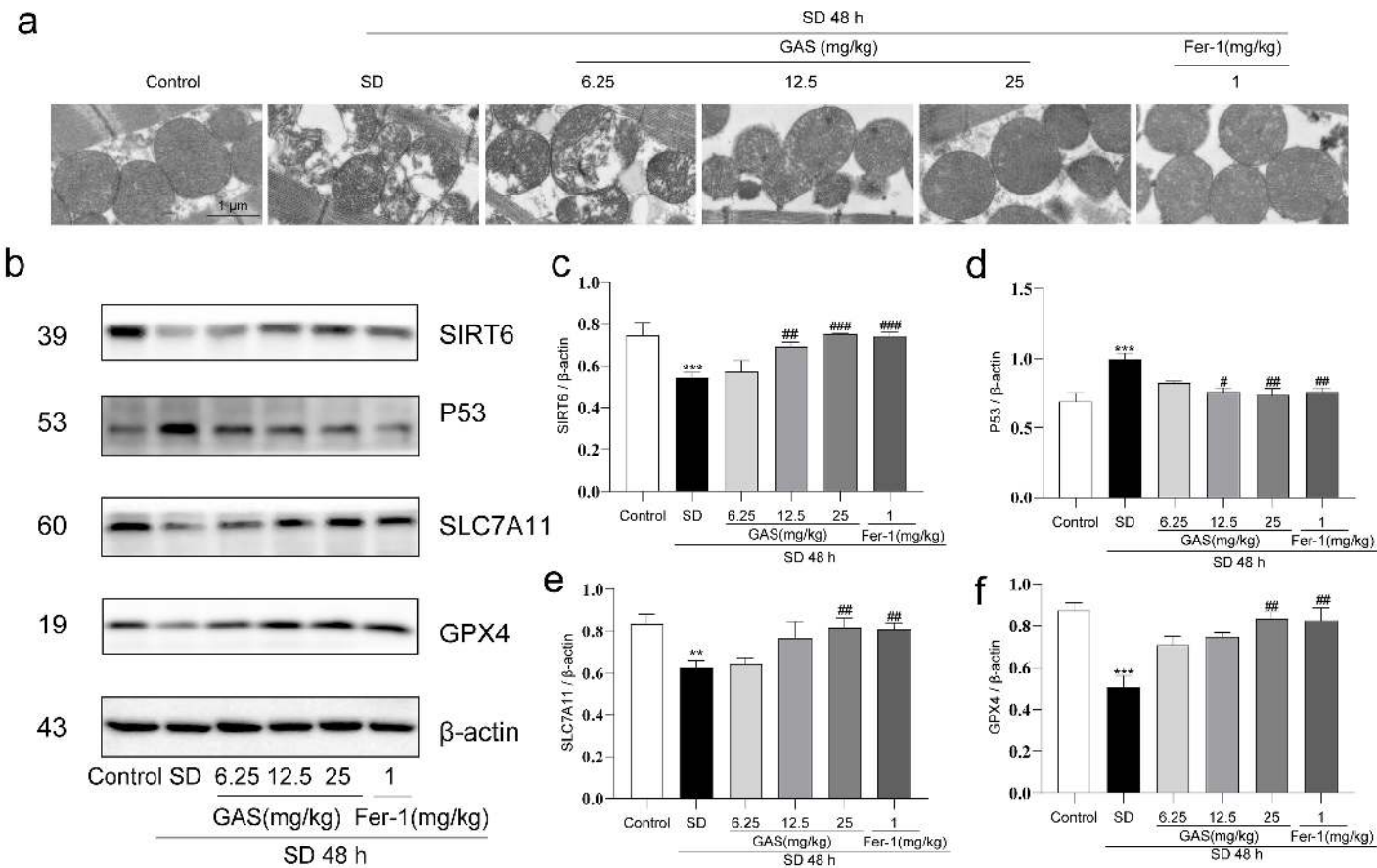


Figure 2

GAS inhibited SD-induced ferroptosis in mice. Transmission electron microscopy. Scale bar: 1 μ m (a). GAS ameliorates myocardial ferroptosis-related protein and SIRT6 changes in sleep deprived mice. (b) Expression bands of individual proteins in mice hearts. (c-f) Gray scale analysis of SIRT6, P53, SLC7A11, GPX4. n=3/group. ** P < 0.01, *** P < 0.001, contrast to the control group. # P < 0.01, ## P < 0.001, ### P < 0.001, contrast to the SD group.

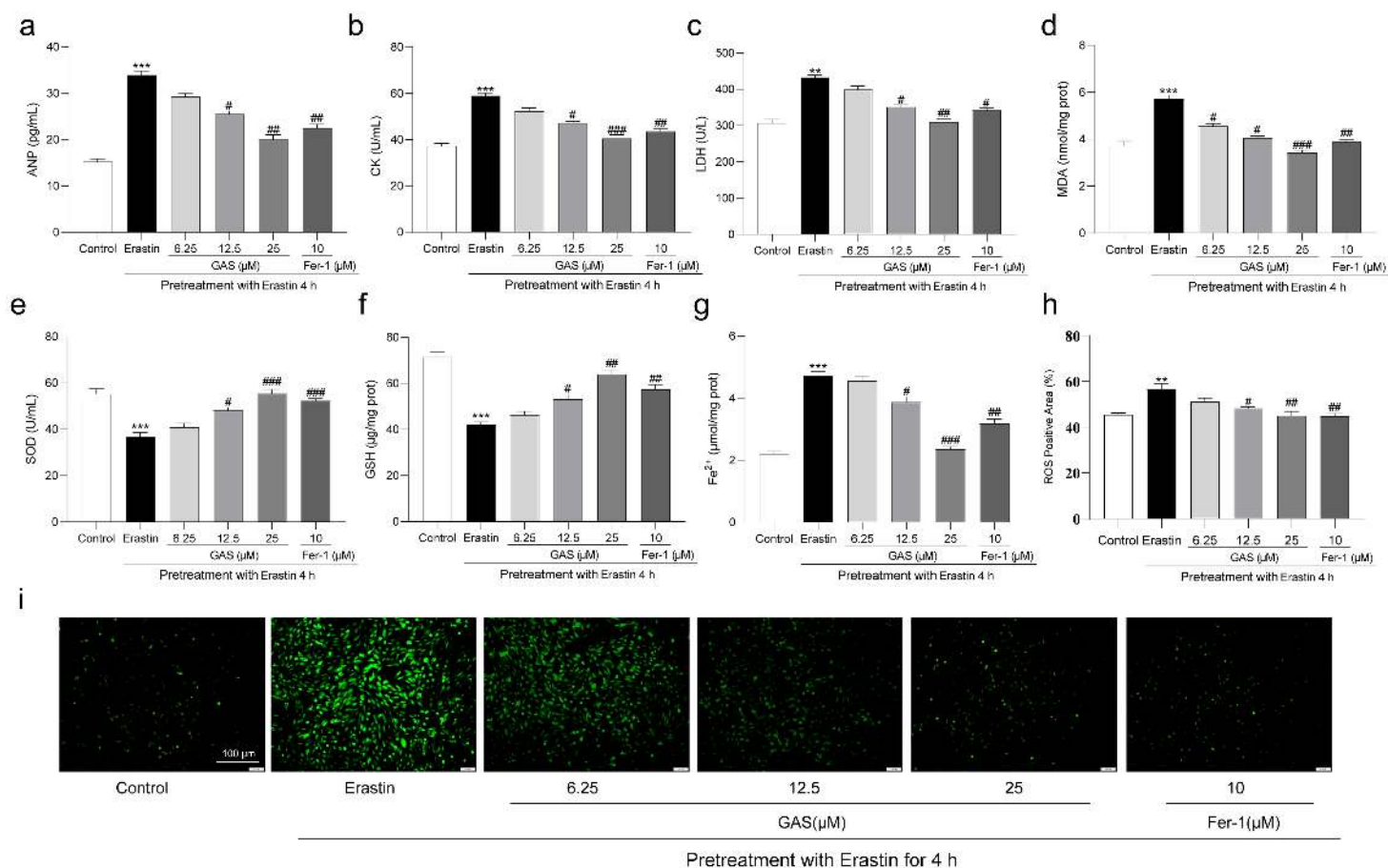


Figure 3

GAS inhibited Erastin-induced ferroptosis in H9c2 cells. GAS inhibited the abnormal changes of ANP (a), CK (b), LDH (c), MDA (d), SOD (e), GSH (f) and Fe²⁺ (g) in H9c2 cells pretreated with Erastin. n=5 / group. (h-i) Detection of ROS levels in H9c2 cells pretreated with Erastin. n= 3/group. Scale bar: 100 μm ***P* < 0.01, ****P* < 0.001, contrast to the control group. #*P* < 0.05, ##*P* < 0.001, ###*P* < 0.001 versus the Erastin group.

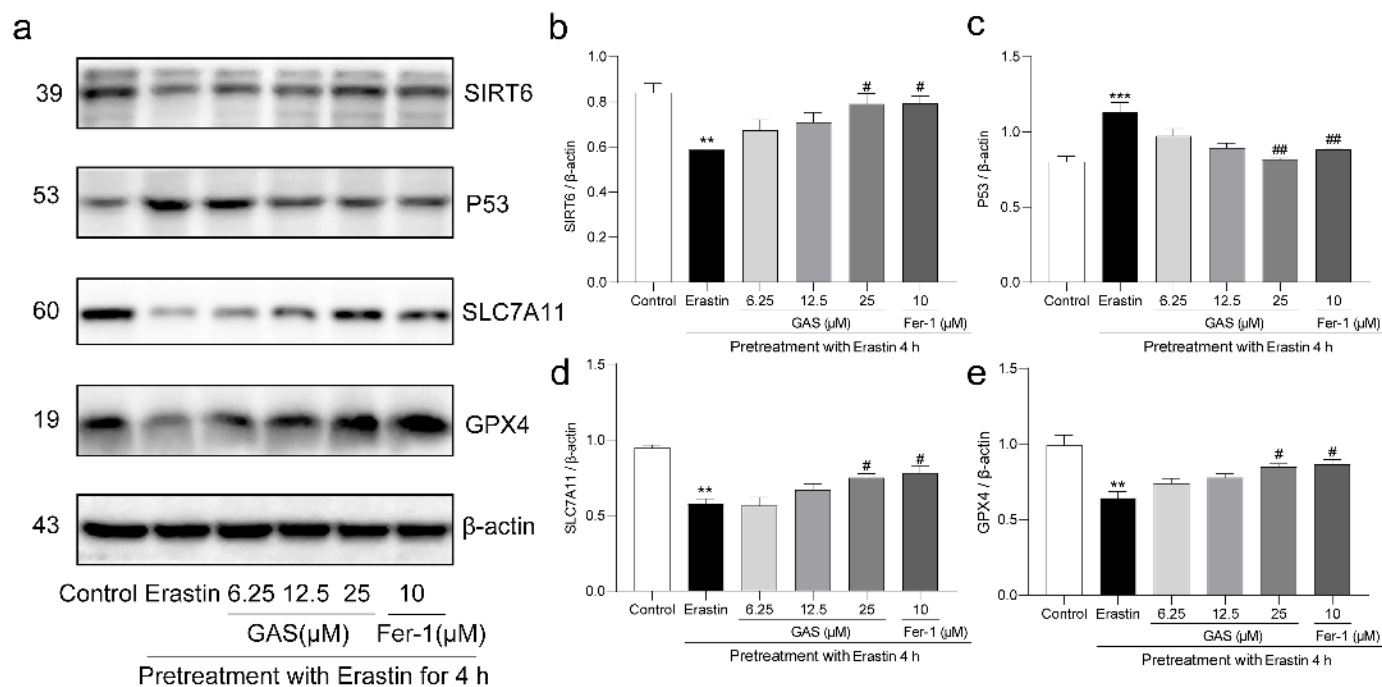


Figure 4

GAS reversed Erastin-induced aberrant expression of SIRT6 and ferroptosis-related proteins in H9c2 cells. (a) Expression bands of individual proteins in H9c2 cells. (b-e) Gray scale analysis of SIRT6, P53, SLC7A11, GPX4. $n = 3$ / group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, contrast to the control group; # $P < 0.05$, ## $P < 0.01$, contrast to the Erastin group.

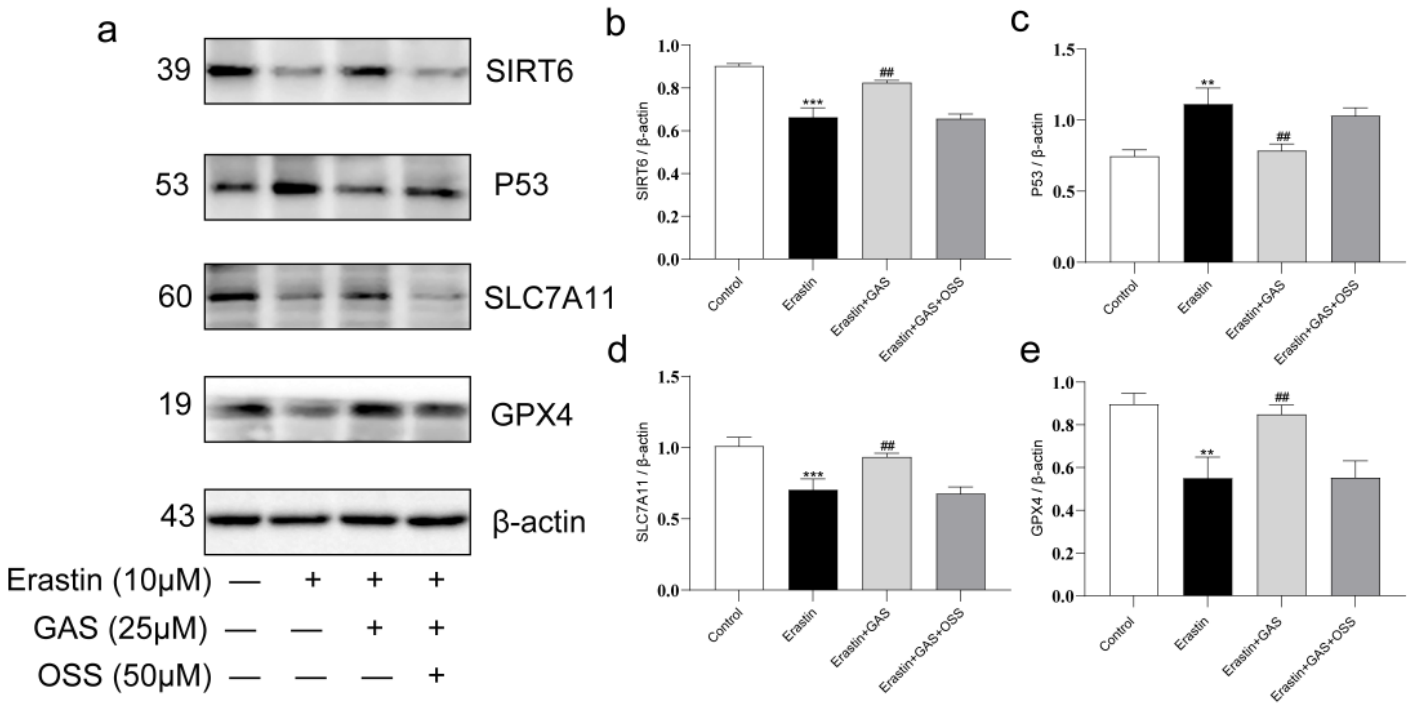


Figure 5

OSS inhibited the therapeutic effect of GAS on ferroptosis in H9c2 cells (a) Bands of individual protein expression in H9c2 cells. (b-e) Gray scale analysis of SIRT6, P53, SLC7A11, GPX4. n = 3 / group. $**P < 0.01$, $***P < 0.001$, contrast to group. $##P < 0.05$, $###P < 0.001$, contrast to the Erastin group.

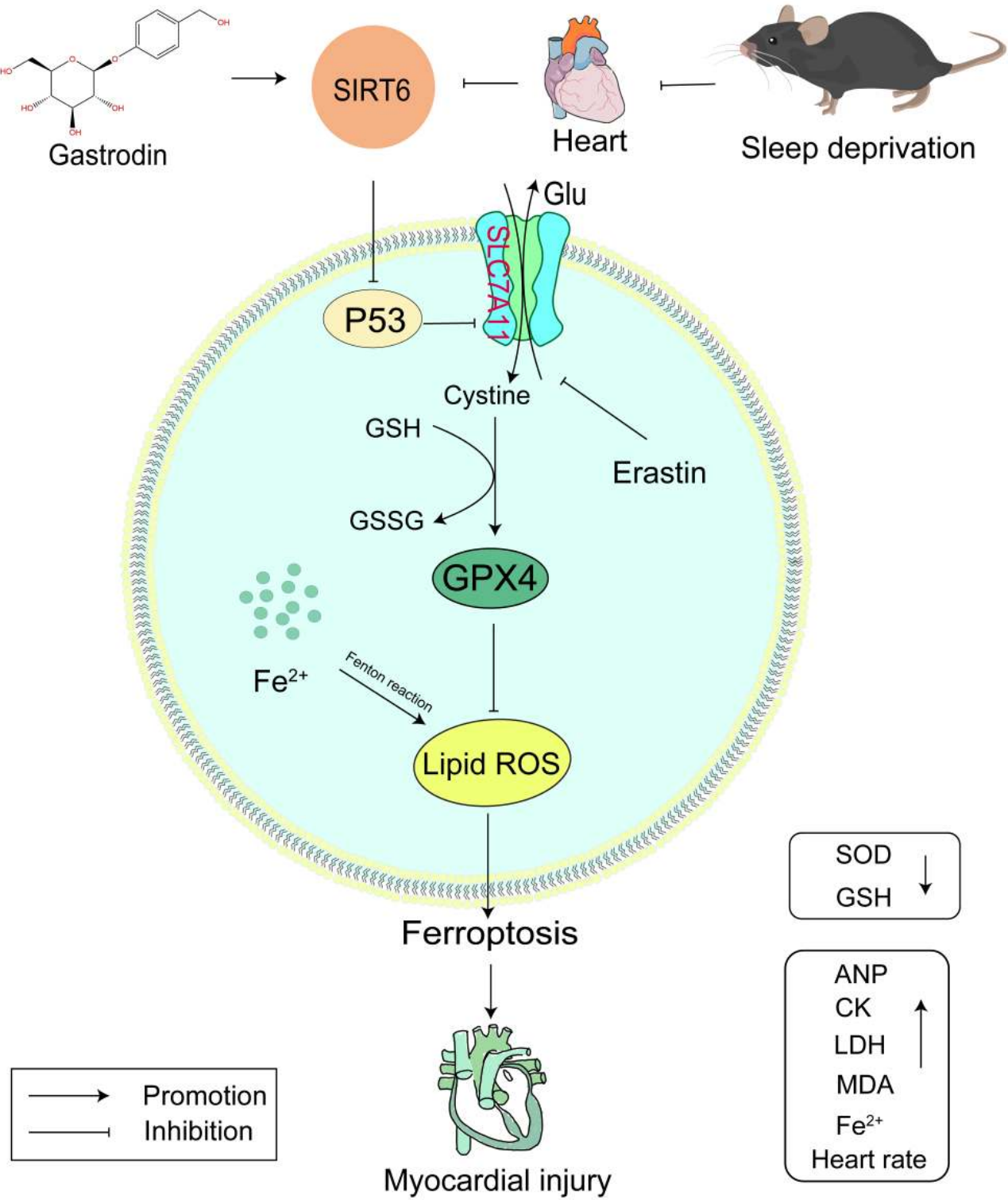


Figure 6

Gastrodin prevents myocardial injury in sleep-deprived mice by suppressing ferroptosis through SIRT6.

Supplementary Files

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