The participation of ferroptosis in fibrosis of the heart and kidney tissues in Dahl salt-sensitive hypertensive rats

The First Affiliated Hospital of University of South China, University of South China

Research Article

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Abstract

Background and Purpose

Hypertension is considered a major risk factor for cardiovascular diseases, and salt-sensitive hypertension is often more prone to induce damage to target organs such as the heart and kidneys. Abundant recent studies have demonstrated a close association between ferroptosis and cardiovascular diseases. Therefore, this study aimed to investigate whether ferroptosis is involved in the occurrence and development of myocardial fibrosis and renal fibrosis in salt-sensitive hypertensive rats, providing new insights into the mechanisms underlying target organ damage in salt-sensitive hypertension.

Methods

Ten 7-week-old male Dahl salt-sensitive (Dahl-SS) rats were randomly divided into two groups after 1 week of adaptation feeding. One group received a regular diet containing 0.3% NaCl (Normal Diet Saline, NDS group), and the other group received a high-salt diet containing 8% NaCl (High Diet Saline, HDS group) for 8 consecutive weeks. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) of Dahl-SS rats were measured and recorded weekly. Structural changes in the heart and kidney tissues of Dahl-SS rats were observed using HE staining and Masson staining. Ultrastructural morphological changes in the heart and kidney tissues of Dahl-SS rats were observed under transmission electron microscopy. Prussian blue staining was used to observe iron deposition in the heart and kidney tissues of Dahl-SS rats. Iron content and malondialdehyde (MDA) content in the heart and kidney tissues of Dahl-SS rats were quantitatively compared using a colorimetric method. Lastly, protein expression of xCT and GPX4 in the heart and kidney tissues of Dahl-SS rats was detected using immunofluorescence and Western blot techniques.

Results

At the end of 8 weeks, compared to the NDS group, rats in the HDS group showed significantly elevated systolic blood pressure (SBP) and diastolic blood pressure (DBP) (P < 0.05). Disordered arrangement of myocardial cells and cell swelling were observed; glomerular solidification, partial renal tubules atrophy, and disordered arrangement were evident. Additionally, collagen fiber deposition significantly increased in the cardiac interstitium, glomeruli, and renal tubular interstitium (P < 0.01). Transmission electron microscopy revealed characteristic changes of ferroptosis in the ultrastructure of the heart and kidney tissues of rats in the HDS group, including reduced or disappeared mitochondria volume, decreased or vanished cristae, and increased density of mitochondrial double membranes. Prussian blue staining confirmed iron deposition in the heart and kidney tissues of rats in the HDS group, accompanied by a significant increase in iron content and MDA levels (P < 0.05). Immunofluorescence and Western blot results both indicated a significant downregulation (P < 0.05) in the expression of proteins associated with inhibiting ferroptosis, xCT, and GPX4 in the HDS group.
Ferroptosis is involved in the damage and fibrosis of the heart and kidney tissues in salt-sensitive hypertensive rats.

Introduction

Hypertension poses an increasing healthcare burden and stands as a major risk factor for cardiovascular diseases, including myocardial infarction, heart failure, renal damage, and stroke\(^1\). Salt-sensitive hypertension (SSH) is the most common type of hypertension in China, with a prevalence ranging from 28–74\(^2\). Patients with SSH exhibit significantly higher urinary microalbumin excretion and left ventricular weight compared to non-salt-sensitive hypertensive patients. Target organ damage occurs earlier, and the incidence and mortality of cardiovascular events are significantly higher in SSH patients\(^3,4\). However, the mechanisms underlying target organ damage in SSH patients remain unclear. Therefore, further exploration of the mechanisms of target organ damage in salt-sensitive hypertension is of great significance.

Research has revealed that various types of cell death, such as necrosis, apoptosis, and autophagy, are involved in the process of target organ damage in hypertension\(^5-7\). In recent years, a novel form of programmed cell death called ferroptosis has been proposed, first discovered by Dr. Brent and colleagues in 2012\(^8\). Ferroptosis is characterized by iron-dependent accumulation of lethal levels of lipid peroxides, manifested by the aggregation of iron ions, particularly ferrous ions, and the accumulation of lipid peroxides\(^9\). Although research on ferroptosis has mainly focused on neurodegenerative diseases, cancer, and brain injury\(^10,11\), recent studies suggest a crucial role for ferroptosis in the progression of cardiovascular diseases\(^12\). Vincenzo et al. demonstrated that Empagliflozin (EMPA) inhibits arsenic-induced cardiomyocyte ferroptosis through NLRP3 and MyD88-related pathways, significantly improving myocardial fibrosis and cardiac function in mice\(^13\). Wang's team revealed that effective improvement of myocardial fibrosis in TAC mice could be achieved by inhibiting MLK3 signal expression in myocardial cells, as MLK3 signaling induces ferroptosis in myocardial cells\(^14\). Cai et al. further found that Alox15/15-HpETE could exacerbate myocardial fibrosis induced by ischemia/reperfusion injury by enhancing cardiomyocyte ferroptosis\(^15\). Thus, there is a close connection between ferroptosis and myocardial fibrosis.

Hypertensive heart disease (HHD) is a cardiovascular disease characterized by progressive thickening of the left ventricle (LV) and pathological remodeling, including myocardial fibrosis, due to sustained high blood pressure\(^16\). The kidneys are another major organ affected by hypertension-related damage. Fibrosis is also a major pathological change in hypertensive kidney disease, characterized by glomerulosclerosis, tubular atrophy, and interstitial fibrosis\(^17\). Hypertensive kidney disease is the second leading cause of chronic kidney disease (CKD) after diabetic nephropathy\(^18\). Cheng et al. found that
Ginkgolide B can protect renal cells from ferroptosis by inhibiting ubiquitination of glutathione peroxidase 4 (GPX4) and thereby improve the progression of renal fibrosis in diabetic rats\[^{19}\].

However, there is no research exploring the relationship between ferroptosis and heart and kidney damage caused by salt-sensitive hypertension, and the potential regulatory mechanisms behind it. Therefore, this study aims to investigate whether ferroptosis is involved in the damage to target organs in salt-sensitive hypertension. This exploration is expected to provide new research directions for understanding the mechanisms of target organ damage in this type of hypertension and offer new targets for future prevention and treatment strategies.

## Materials and Methods

### Materials

High-salt feed containing 8% NaCl was purchased from Jiangsu Synergy Pharmaceutical Biotechnology Co., Ltd. (Jiangsu, China). HE and Masson staining kits were obtained from Boster Biological Technology Co., Ltd. (Wuhan, China). Prussian blue iron staining test kit (G1029) was obtained from Servicebio Technology Co., Ltd. (Wuhan, China). Malondialdehyde (MDA) (A003-1) and tissue iron content detection kit (A039-2) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Glutathione peroxidase 4 (GPX4) monoclonal antibody (67763-1-Ig) was obtained from Protrintech Co., Ltd. (Wuhan, China). Amino acid transport system xc-xCT antibody (bs-6883R) was obtained from Beijing Boaosen Biotechnology Co., Ltd. (Beijing, China). TRITC-labeled goat anti-rabbit IgG fluorescent secondary antibody (BA1142), FITC-labeled goat anti-mouse IgG fluorescent secondary antibody (BA1126), and DAPI staining solution (AR1176) were obtained from Boster Biological Technology Co., Ltd. (Wuhan, China).

### Experimental Animals and Model Establishment

The animal experiments were approved by the Animal Welfare and Ethics Review Committee of South China University (Animal license number: SCXK (Jing) 2016-0006, SPF quality qualification certificate: No.11001121104248861). Ten SPF-grade 7-week-old male Dahl salt-sensitive rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), with a weight of 240 ± 10g and in good health. All rats were housed in the SPF-grade breeding room (constant temperature of 25°C, relative humidity of 45–65%), with free access to food and water, and subjected to a 12-hour light-dark cycle. After one week of adaptive feeding, the rats were randomly divided into two groups: one group received a normal diet (0.3% NaCl, n = 5, NDS group), and the other group received a high-salt diet (8% NaCl, n = 5, HDS group) for 8 weeks. Blood pressure and body weight of the rats were monitored weekly.

### Blood Pressure Measurement

Within the first week of adaptive feeding, the rats underwent adaptive training for tail artery blood pressure measurement using the medlab rat non-invasive blood pressure measurement analysis system.
purchased from Kalstein Biological Technology Co., Ltd. (Nanjing, China). Blood pressure was measured and recorded for each rat before grouping. After grouping, blood pressure was measured weekly at the same time (8–10 am), with three repeated measurements. Rats rested for 10–15 minutes between measurements, and the data were averaged.

**Tissue Sample Collection**

At the end of the 8th week, all rats were euthanized, and the hearts and kidneys were immediately collected. Tissue samples were fixed according to the requirements of each experimental condition for subsequent experiments.

**Transmission Electron Microscopy (TEM)**

The heart and kidney tissues of rats were fixed in 5% glutaraldehyde, cut into 2mm×2mm slices, washed with PBS buffer, fixed in 1% osmium tetroxide, dehydrated in a gradient of ethanol, embedded, sliced into 70 nm sections, and stained with uranyl acetate and lead nitrate. The ultrastructure of tissue cells was observed under a transmission electron microscope.

**Histological Analysis**

The heart and kidney tissues of rats were fixed in 4% paraformaldehyde, dehydrated in a gradient of ethanol, and embedded in paraffin. The 4µm sections were stained with HE and Masson according to the instructions of the staining kit. Tissue collagen volume fraction was semi-quantitatively assessed using Image J software.

**Prussian Blue Staining**

The heart and kidney tissues of rats were fixed in 4% paraformaldehyde, dehydrated in a gradient of ethanol, and embedded in paraffin. The 4µm sections were stained with Prussian blue according to the instructions of the staining kit. Images were captured and analyzed.

**Immunofluorescence Staining**

The heart and kidney tissues of rats were fixed in 4% paraformaldehyde, dehydrated in a gradient of ethanol, and embedded in paraffin. The 4µm sections were subjected to antigen retrieval and blocked with 5% BSA. Then, the sections were incubated overnight at 4°C with GPX4 monoclonal antibody (1:400) and xCT antibody (1:100). After washing with PBS, the sections were incubated with the corresponding fluorescent secondary antibodies, followed by DAPI staining. Fluorescence reaction area and intensity of GPX4 and xCT were analyzed using Image J software.

**Biochemical Analysis**

Heart and kidney tissues were accurately weighed, and 10% tissue homogenates were prepared in 0.9% physiological saline. The protein concentration was quantified using the BCA protein assay kit. Tissue iron content and MDA content were determined according to the instructions of the MDA test kit and tissue iron test kit.
Western Blot Analysis

Rat heart and kidney tissues were lysed with RIPA lysis buffer. After centrifugation, the supernatant was collected, and the protein concentration was quantified and adjusted using the BCA protein assay kit. The samples were denatured at 95°C for 10 minutes, separated in an 8–12% polyacrylamide gel, and transferred to a PVDF membrane. After blocking with 5% skim milk, the membrane was incubated overnight at 4°C with GPX4 antibody (1:1000) and xCT antibody (1:1000). After washing with Tween 20-containing Tris-buffered saline (TBST), the membrane was incubated with the corresponding secondary antibodies at room temperature for 1 hour. The target bands were visualized using an enhanced chemiluminescence reagent, and quantitative analysis was performed using Image J software.

Statistical Analysis

Data were analyzed using SPSS 26.0. The Shapiro-Wilk test was used to determine whether the data in each group followed a normal distribution. Quantitative data were expressed as mean ± standard deviation (x ± S). The t-test was used for comparisons between two groups, and P < 0.05 was considered statistically significant. Graphs were generated using GraphPad Prism 9.0.

Results

Effect of High-Salt Diet on Blood Pressure in Dahl Salt-Sensitive Rats

During the high-salt diet feeding period, SBP and DBP of both groups of rats were measured for 8 weeks. Starting from the 2nd week, the SBP and DBP of rats in the HDS group were significantly higher than those in the NDS group. Until the end of the 8th week, both SBP and DBP of rats in the HDS group were significantly higher than those in the NDS group (P < 0.05) (Fig. 1, Table 1).

<table>
<thead>
<tr>
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<th>NDS (n = 5)</th>
<th>HDS (n = 5)</th>
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<tr>
<td>SBP (mmHg)</td>
<td>132.91 ± 6.48</td>
<td>206.48 ± 4.09*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>86.57 ± 3.61</td>
<td>154.75 ± 6.16*</td>
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Note: Values are expressed as mean ± standard deviation. Abbreviations: NDS, rats in the normal diet group; HDS, rats in the high-salt diet group. P < 0.05, compared to the NDS group.

Structural Changes in the Heart and Kidney Tissues of Dahl Salt-Sensitive Rats
Heart Tissue - HE Staining and Masson Staining, In the NDS group, myocardial cells were neatly arranged with tight intercellular connections, clear boundaries, and uniform cell nuclei. The myocardial fibers were organized in an orderly manner. In the HDS group, myocardial cells showed disorganized arrangement, widened intercellular gaps, and some cell boundaries became unclear, accompanied by cell swelling. Additionally, irregular cell nuclei and inflammatory cell infiltration in the myocardial interstitium were observed. There was a significant proliferation of collagen fibers in the myocardial interstitium and surrounding vascular areas in the HDS group, distributed in a loose and disordered manner. Semi-quantitative analysis of collagen fiber volume in the myocardial tissues of both groups showed a significant increase in the HDS group compared to the NSD group (P < 0.01) (Fig. 2a, c, e).

Kidney Tissue - HE Staining and Masson Staining, In the NDS group, the structure of renal glomeruli, tubules, and interstitium was clear and normal, with no significant deposition of collagen fibers. However, in the HDS group, glomerular contraction was observed, and segmental fibrinoid necrosis occurred in some glomerular capillaries. The intima of afferent arterioles in the glomeruli thickened, and the lumen narrowed. Some renal tubules appeared atrophied, with disordered arrangement and significant infiltration of inflammatory cells in the interstitium. Blue-stained collagen fibers were observed in the glomeruli, tubules, and interstitium, indicating a significant increase in fibrous proliferation. Semi-quantitative analysis of collagen fiber volume in the kidney tissues of both groups showed a significant increase in the HDS group compared to the NDS group (P < 0.01) (Fig. 2b, d, f).

Expression of Ferroptosis-Related Markers in the Heart and Kidney Tissues of Dahl Salt-Sensitive Rats

Transmission electron microscopy was employed to observe the ultrastructural changes in the heart and kidney tissues of the two rat groups. In comparison to the NDS group, rats in the HDS group exhibited characteristic features such as reduced mitochondrial volume, increased density of the mitochondrial double membrane, and a decrease or disappearance of cristae in both heart and kidney tissues (shown in Fig. 3c, d). Prussian blue staining revealed a significant deposition of blue-stained particles in the heart and kidney tissues of rats in the HDS group (shown in Fig. 3a, b). The results of colorimetric assays demonstrated higher iron and MDA content in the heart and kidney tissues of rats in the HDS group compared to the NDS group (P < 0.05) (shown in Fig. 3e-h). Immunofluorescence results indicated a notable decrease in the fluorescence intensity of xCT (marked in red) and GPX4 (marked in green) in the heart and kidney tissues of rats in the HDS group compared to the NDS group (P < 0.05) (shown in Fig. 4a-d). Western blot analysis further revealed a significant downregulation of protein expression levels of xCT and GPX4 in the heart and kidney tissues of rats in the HDS group compared to the NDS group (P < 0.05) (shown in Fig. 4e-h).

Discussion

The clinical characteristics of salt-sensitive hypertension mainly involve a significant increase in blood pressure after prolonged salt loading. The kidneys, as target organs for hypertension, undergo early
damage. As the condition progresses, there is a gradual increase in left ventricular mass, leading to structural changes in the heart. Studies have indicated that the long-term survival rate of patients with salt-sensitive hypertension is significantly lower than that of non-salt-sensitive hypertensive patients\cite{20}. In this study, we successfully established a salt-sensitive hypertension rat model using an 8-week high-salt diet intervention. Histopathological examination using HE and Masson staining revealed pathological damage and fibrotic changes in the heart and kidney tissues of rats in the high-salt diet (HDS) group.

Myocardial fibrosis is a central pathological change in hypertensive heart disease (HHD), characterized by excessive deposition of extracellular matrix fibrous proteins in the myocardium and surrounding blood vessels. This fibrous protein accumulation disrupts the normal structure of the myocardium, leading to disordered arrangement of myocardial cells\cite{21}. Our experimental observations revealed varying degrees of pathological damage in the hearts of the HDS group rats. Specifically, myocardial cells showed significant hypertrophy, disordered arrangement, increased intercellular spacing, and irregular nuclear morphology. The myocardial interstitium exhibited a significant fibrotic change with abundant infiltration of inflammatory cells and pronounced fibrosis in the vicinity of blood vessels. Our findings are consistent with the results reported by Sawano et al.\cite{22}.

After 8 weeks of a high-salt diet, structural damage to the renal units was observed in the HDS group, with glomerular contraction and sclerosis, partial atrophy, and disordered arrangement of renal tubules. Additionally, there was significant infiltration of inflammatory cells in the renal tissue interstitium, along with a noticeable increase in collagen fibers in both the interstitium and glomeruli, characteristic of fibrosis. Our results align with the findings of Eri Manabe et al., who detected tubular damage and increased glomerulosclerosis index in Dahl salt-sensitive hypertensive rats using periodic acid-Schiff staining\cite{23}.

Ferroptosis, a new form of iron-dependent, lipid peroxidation-driven cell death, differs from traditional cell death mechanisms such as necrosis, apoptosis, and autophagy in that it lacks obvious morphological changes, such as membrane rupture, apoptotic bodies, or autophagic vacuoles. Instead, ferroptosis’s morphological features primarily manifest as characteristic alterations in mitochondria: reduced mitochondrial volume, increased density of the mitochondrial double membrane, decreased or disappearing cristae, and outer membrane rupture\cite{25}. Transmission electron microscopy in our study revealed characteristic changes indicative of ferroptosis in some mitochondria of the heart and kidney tissues of the HDS group rats relative to the normal diet (NDS) group. Thus, we speculate that ferroptosis may contribute to organ damage in salt-sensitive hypertension.

The initiation of ferroptosis depends on the presence and accumulation of iron ions, where iron overload is considered a key mechanism. High iron intake not only leads to severe cardiac damage but may also induce hypertrophic cardiomyopathy. Application of iron chelators significantly improves myocardial remodeling\cite{26}. Within cells, Fe$^{2+}$ is highly unstable and rapidly reacts with hydrogen peroxide (H$_2$O$_2$) via the Fenton reaction, generating reactive oxygen species (ROS) and forming lipid peroxides, with
malondialdehyde (MDA) being a major product of these reactions \[^{27}\]. Prussian blue staining in our experiment revealed iron deposition in the heart and kidney tissues of the HDS group rats. Further biochemical assays confirmed significantly elevated iron and MDA levels in the heart and kidney tissues of the HDS group compared to the NDS group. This substantiates iron overload-induced ferroptosis involvement in heart and kidney organ damage in salt-sensitive hypertension.

In addition to iron overload, the xCT/GPX4 signaling pathway plays a crucial regulatory role in cellular ferroptosis. xCT is responsible for transporting cysteine into cells, leading to glutathione (GSH) synthesis. Continuous activation of GSH sustains GPX4 \[^{28}\], which converts lipid peroxides into non-toxic lipid alcohols, thereby inhibiting cellular ferroptosis. Studies have shown that resveratrol inhibits ferroptosis by upregulating the xCT/GPX4 signaling pathway, reducing myocardial damage, and improving myocardial fibrosis after myocardial infarction \[^{29}\]. Furthermore, Zhu et al. found that flavonoid drugs can inhibit ferroptosis and delay the progression of renal fibrosis, and their mechanism of action is closely related to the increased expression of xCT and GPX4 proteins \[^{30}\].

In this study, immunofluorescence observations revealed significantly lower fluorescence intensities of xCT and GPX4 in the heart and kidney tissues of the HDS group rats. Western blot analysis further confirmed relatively lower protein expression levels of xCT and GPX4 in the HDS group. When salt-sensitive hypertension-induced target organ damage occurs in Dahl salt-sensitive rats, inhibited xCT activity and reduced GPX4 expression are observed. Thus, we speculate that suppressed xCT activity and decreased GPX4 expression may be one of the mechanisms by which ferroptosis contributes to heart and kidney damage and fibrosis in salt-sensitive hypertension.

Our study provides preliminary insights into the involvement of ferroptosis in target organ damage in salt-sensitive hypertension. However, the application of ferroptosis-specific inhibitors has not been explored. To further elucidate the correlation between ferroptosis and fibrosis in heart and kidney tissues of Dahl salt-sensitive hypertensive rats, future research should involve intervention experiments using ferroptosis inhibitors. In subsequent studies, we will continue to investigate the specific mechanisms of the xCT/GPX4 signaling pathway in the target organ damage caused by salt-sensitive hypertension.

**Conclusion**

The heart and kidney tissues of Dahl salt-sensitive hypertensive rats exhibit characteristic mitochondrial changes associated with ferroptosis. The expression of proteins closely related to ferroptosis, xCT, and GPX4, is significantly downregulated in the heart and kidney tissues. This discovery suggests that ferroptosis may contribute to target organ damage in salt-sensitive hypertension. In-depth exploration of the role and impact of ferroptosis in salt-sensitive hypertension will provide further insights into the understanding, diagnosis, and treatment of target organ damage, paving the way for new research directions and treatment targets.

**Declarations**
Author Contributions

Y.-Q.H. and J.W. developed the study concept and design, analyzed and interpreted the data, and drafted the manuscript. Y.-Q.H., J.W., K.P., J.Y., H.-L.C., P.-Y.J., Y.-F.D., X.L. and S.-L.Z. performed experiments; Y.-Q.H. and J.W. analyzed and interpreted the data. J.Y., H.-L.C., P.-Y.J., Y.-F.D., X.L. and S.-L.Z. provided reagents/materials/analysis tools. All authors read and approved the final manuscript.

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

The authors declare that they have no conflicts of interest.

References

2. [J]., 2023; 51(4) : 364-376.

Figures

**Figure 1**

Continuous measurement of blood pressure in both groups of rats for 8 weeks. (a) SBP. (b) DBP. Abbreviations: NDS, rats in the normal diet group; HDS, rats in the high-salt diet group. *p<0.05, compared to the NDS group.
Figure 2

Pathological Examination of Heart and Kidney Tissues in Dahl Salt-Sensitive Rats. (a-b) HE staining of heart and kidney tissues in both groups, with scale bars representing 100μm and 50μm, respectively. (c-d) Masson staining of heart and kidney tissues in both groups, with scale bars representing 100μm and 50μm, respectively. (e-f) Comparison of collagen fiber content in heart and kidney tissues between the two groups, n=5. Values are expressed as mean ± standard deviation. Abbreviations: NDS, rats in the normal diet group; HDS, rats in the high-salt diet group. **P<0.01, compared to the NDS group.
Figure 3

Comparison of Prussian Blue Staining, Ultrastructural Electron Microscopy, Tissue Iron Content, and MDA Content in Dahl Salt-Sensitive Rats. 

a. Prussian blue staining of heart tissues in both rat groups. b. Prussian blue staining of kidney tissues in both rat groups. The scale bar represents 100μm. (c-d). Ultrastructural changes in mitochondria were observed under electron microscopy in the heart and kidney tissues of both rat groups. Red arrows indicate reduced mitochondrial volume, increased density of the mitochondrial double membrane, and decreased or disappeared cristae. Scale bars represent 1μm, 200nm, and 500nm. (e-f). Comparison of iron content and MDA content in heart tissues of both rat groups, n=5. (g-h). Comparison of iron content and MDA content in kidney tissues of both rat groups, n=5. Values are expressed as mean ± standard deviation. Abbreviations: NDS, normal diet group; HDS, high-salt diet group. *P<0.01, compared to the NDS group.
Figure 4

Protein Expression Levels of xCT and GPX4 in Heart and Kidney Tissues of Dahl Salt-Sensitive Rats. (a-b). Confocal immunofluorescence images of xCT (red), GPX4 (green), and cell nuclei (blue) in heart and kidney tissues of both rat groups. Scale bar represents 50μm. (c-d). Semi-quantitative results of immunofluorescence staining intensity for xCT and GPX4 in heart and kidney tissues of both rat groups, n=5. (e-f). Western blot analysis of protein expression levels of xCT and GPX4 in heart and kidney tissues.
of both rat groups, n=3. (g-h). Average grayscale values of xCT and GPX4 in heart and kidney tissues of both rat groups, n=3. Values are expressed as mean ± standard deviation. Abbreviations: NDS, normal diet group; HDS, high-salt diet group. *P<0.05, compared to the NDS group.