

Salidroside inhibits renal ischemia/reperfusion injury-induced ferroptosis by the PI3K/AKT signaling pathway

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Abstract. Renal ischemia/reperfusion injury (RIRI) represents the principal factor underlying acute kidney injury (AKI), which primarily stems from cellular injuries and ferroptosis caused by reactive oxygen species (ROS). Salidroside (SA), an antioxidant natural ester, has been attributed with the potential to protect against RIRI. In the present study, rats received daily SA doses (1, 10, or 100 mg/kg) by gavage for 7 consecutive days before surgery. The results revealed aggravated renal injury in the RIRI group, which was effectively prevented by SA pretreatment (10 and 100 mg/kg), with the 1 mg/kg dosage demonstrating lesser efficacy. Additionally, the results indicated that SA pretreatment mitigated the RIRI-related upregulation of antioxidative superoxide dismutase. *In vitro* studies corroborated SA's ability to maintain hypoxia/reoxygenation-treated NRK cell viability, with the protective effect being observed at SA concentrations $\geq 1 \mu\text{M}$ and peaking at $100 \mu\text{M}$. Furthermore, the results showed that SA safeguarded renal tubular epithelial cells from oxidative damage, reduced ROS accumulation, and inhibited ferroptosis via activation of the PI3K/AKT signaling pathway. Therefore, the results of the present study highlight the promising therapeutic potential of SA as an effective intervention for RIRI via targeting of PI3K/AKT signaling pathway-mediated anti-oxidative and anti-ferroptotic mechanisms.

Introduction

Ischemia-reperfusion injury (IRI) is a frequent occurrence seen in various diseases and surgical procedures such as coronary heart disease, kidney transplants, and liver transplants (1-3). Interruption or reduction of blood flow leads to cellular

ischemia and hypoxia, resulting in tissue and/or organ damage. The metabolites and mediators produced during ischemia promote further damage, as reflected by the serious dysfunction of the cell ultrastructure, metabolism, electrophysiology, and function (4). The primary causes of renal ischemia-reperfusion injury (RIRI) are cardiac arrest (systemic hypoperfusion) and renal surgical intervention (5). In the case of kidney transplants, kidney injury occurs following a short period of warm ischemia following the removal of the organ from the donor, a prolonged period of cold ischemia during cryopreservation, and a final stage of warm ischemia during recipient implantation. Post-revascularization, renal blood reperfusion triggers a series of events that can exacerbate renal injury (6), resulting in hemodynamic fluctuations, inflammation, and damage to tubular epithelial cells (7). The pathogenesis of RIRI may be attributed to structural and functional dysmetabolism of the kidneys induced by a range of factors, such as excessive production of oxygen-free radicals, intracellular calcium overload, inflammatory factors and transmitters, apoptosis, membrane lipid peroxidation, and alterations in nitric oxide content (8-10).

Salidroside (SA) is a natural active ingredient extracted from *Rhodiola rosea* (11), renowned for its diversified biological activities, including anti-inflammatory, anti-oxidative, anti-tumorigenic, and anti-radiation properties (11). As a result of its broad pharmacological effects, SA has attracted the attention both domestically and internationally. Numerous studies have documented the protective effects of SA against a range of organ injuries, primarily by reducing oxidative stress, inhibiting apoptosis, reducing intracellular calcium overload, and enhancing mitochondrial function (11-13). Prior studies have shown SA to have a protective effect on myocardial cell injury caused by hypoxia, as well as having anti-arrhythmic properties. Furthermore, it inhibits the proliferation and contraction of vascular smooth muscle cells. Its mechanisms possibly involve reducing creatine phosphate (CK) activity and malonaldehyde (MDA) content, increasing superoxide dismutase (SOD) activity, increasing sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA) activity, reducing Ca^{2+} overload, and suppressing apoptosis-related gene expression (14-16). Although numerous studies have confirmed that SA has a protective effect on IRI of myocardium, testis, and the brain, its effect on RIRI and the underlying mechanisms remain unknown.

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Materials and methods

Animals. Male Sprague-Dawley rats (weighing 200–220 g) were purchased from the Animal Center of Wuhan University. The rats were housed in a temperature and humidity-controlled environment with a 12-h light/dark cycle and provided with standard food and water. This study was performed in accordance with the Helsinki Declaration II (17) and was approved by the Institutional Review Boards of Renmin Hospital of Wuhan University (approval no. K2021-08-012).

Establishment of the RIRI model. The rats were subjected to an 8 h fast prior to the surgical procedure. Thereafter, sodium pentobarbital (50 mg/kg, intraperitoneal injection) was injected into the abdominal cavity to anesthetize the rats, and the limbs were immobilized during the operation. An abdominal incision was used to reveal the renal pedicle, and the bilateral renal arteries were ligated for 45 min. During reperfusion, the hemostatic clamp was removed, and changes in kidney color were assessed. Blood flow to the kidney generally resumed to normal within a few min, which was then followed by suturing of the incision. The sham group underwent the same surgical procedure without clamping of the renal pedicle. After 24 h, rats were humanely sacrificed under anesthesia by intraperitoneal injection with 1% pentobarbital sodium (150 mg/kg). The kidneys were harvested, and serum samples were collected.

In vivo treatment protocols. All rats were randomly divided into three groups: Sham group (n=6), IR group (n=6), and IR+SA group (n=18). To explore the effects of SA on RIRI, SA group rats were given 1, 10, or 100 mg/kg SA (cat. no. 10338-51-9; MedChemExpress) daily by gavage for 7 days before the IR procedure (n=6 per group).

Cell culture. The rat renal tubular epithelial cell line, NRK cells, were cultured in DMEM (cat. no. 12430112; Sigma-Aldrich; Merck KGaA; Thermo Fisher Scientific, Inc.) with 1% Penicillin-Streptomycin solution (cat. no. 15140122; Sigma-Aldrich; Merck KGaA; Thermo Fisher Scientific, Inc.) and 10% FBS (cat. no. 16140089; Sigma-Aldrich; Merck KGaA; Thermo Fisher Scientific, Inc.) and maintained in a humidified incubator at 37°C incubator supplied with 5% CO₂.

Establishment of the hypoxia-reoxygenation (HR) model. NRK cells were starved in serum-free DMEM for 12 h, and then incubated in a thermostatic tri-gas incubator supplied with 95% N₂ for 8 h to simulate a hypoxic environment. Subsequently, the cells were moved to a normoxic incubator for a period of 12 h, followed by the replacement of the media with supplemented with DMEM for 12 h of reoxygenation.

In vitro treatment protocols. To explore the effects of SA and the PI3K/AKT signaling pathway, NRK cells were incubated with SA (1–1,000 μM) or PI3K/AKT-IN-1 (2.62 μM; cat. no. HY-144806; MedChemExpress) at the onset of reoxygenation. Cells were randomly divided into one of four groups: Control group, HR group, HR+SA group, and HR+SA+PI3K/AKT-IN-1 group.

Cell viability assay. Cell viability was determined using a CCK-8 assay (cat. no. C0038; Sigma-Aldrich; Merck KGaA Institute of Biotechnology). Briefly, NRK cells were seeded into 96-well plates at a density of 1×10⁴ cells/well and incubated for 12 h in a thermostatic incubator. Upon treating cells with SA and/or PI3K/AKT-IN-1 for 4 h, 10 μl CCK-8 solution was added to each well, and cells were cultured for an additional 1 h. Subsequently, the absorbance at 450 nm was measured using a microplate reader (PerkinElmer; Thermo Fisher Scientific, Inc.).

Renal function analysis and histopathological analysis. Renal function was evaluated by measuring serum creatinine (Scr) and blood urea nitrogen (BUN) levels in plasma at the Department of Clinical Laboratories of Renmin Hospital, Wuhan University. Kidney samples were procured, fixed with paraformaldehyde, and then embedded in paraffin. The samples were sectioned into 4-μm thick slices, and then stained with hematoxylin for 5 min and eosin for 3 min at room temperature. The severity of RIRI was assessed using the Paller scoring system (18), with histopathological changes graded as follows: i) 1 point, tubular epithelial smoothness or tubular expansion; ii) 1 or 2 points, loss of brush-like edge; iii) 1 or 2 points, obstruction of the tubular lumen; iv) 1 point, cytoplasmic vacuolization; and v) 1 point, cell necrosis. The histological data were analyzed and graded independently by two observers who were blinded to the experimental groups.

Western blotting. Proteins from kidney tissues or NRK cells were extracted and lysed in RIPA buffer supplemented with protease and phosphatase inhibitors. Protein content was measured using a BCA kit (cat. no. A045-4-2, Nanjing Jiancheng Bioengineering Institute). Subsequently, 50 μg protein samples were loaded on 10% SDS gels, resolved using SDS-PAGE, and subsequently transferred to a PVDF membrane. The membranes were blocked with 5% BSA for 1 h at room temperature and incubated with one of the following primary antibodies: Rabbit anti-GPX4 (1:1,000; cat. no. 67763-1-Ig, Sigma-Aldrich; Merck KGaA Group, Inc.), rabbit anti-ACSL4 (1:1,000, cat. no.22401-1-AP; Sigma-Aldrich; Merck KGaA Group, Inc.), rabbit anti-PI3K antibody (1:1,000; cat. no. 4249; Cell Signaling Technology, Inc.), rabbit anti-p-PI3K antibody (1:1,000; cat. no. 17366; Cell Signaling Technology, Inc.), rabbit anti-AKT (1:1,000; cat. no. 9272; Cell Signaling Technology, Inc.), rabbit anti-p-AKT antibody (1:1,000; cat. no. 4060; Cell Signaling Technology, Inc.), rabbit anti-SOD1 antibody (1:1,000; cat. no. 37385; Cell Signaling Technology, Inc.), rabbit anti-SOD2 antibody (1:1,000; cat. no. 13141; Cell Signaling Technology, Inc.) and rabbit anti-GAPDH antibody (1:10,000; cat. no. 5174; Cell Signaling Technology, Inc.) overnight at 4°C. The following day, the membranes were washed in TBST and incubated for 2 h at room temperature with the corresponding secondary antibodies. Signals were visualized using an ECL system and ECL kit (cat. no. W028-2-1; Nanjing Jiancheng Bioengineering Institute), and densitometry analysis was calculated using ImageJ version 1.8.0 (National Institutes of Health).

Detection of the oxidative stress indicators. The levels of malondialdehyde (MDA) and superoxide dismutase (SOD) in kidney tissues or NRK cells were detected using an MDA

assay kit (cat. no. S0131S; Sigma-Aldrich; Merck KGaA Institute of Biotechnology) and SOD assay kit (cat. no. S0101S; Sigma-Aldrich; Merck KGaA Institute of Biotechnology) according to the manufacturer's protocol.

Measurement of ROS levels in the kidney tissues. The levels of ROS were analyzed in the tissues utilizing the dihydroethidium (DHE) fluorescent probe (cat. no. D7008; MilliporeSigma), according to the manufacturer's protocol. The sections were stained by incubation with 50 μ M DHE for 1 h in the dark at room temperature, and then stained with 1 mg/ml DAPI for 10 min at 4°C. The sections were rinsed three times with PBS. A fluorescent microscope (magnification, x200) was used to evaluate the fluorescence intensity at an excitation wavelength of 525 nm and an emission wavelength of 610 nm.

Evaluation of intracellular lipid hydroperoxide (LPO). C11-BODIPY^{581/591} (cat. no. HY-D1301; MedChemExpress, Inc.) was used as a fluorescent probe to assess intracellular LPO levels. The probe integrates into the lipid membrane and is oxidized by intracellular LPO, fluorescing green once oxidized. A total of 5 μ M C11-BODIPY^{581/591} was used, and kidney slices were incubated for 30 min at 4°C. After washing with PBS, the slices were examined by flow cytometer BD FACSAria II (version 7.6.1, BD Biosciences).

Evaluation of ROS levels in NRK cells. The 2',7'-dichlorofluorescein diacetate (DCFH-DA) fluorescent probe (cat. no. D6883; MilliporeSigma-Aldrich; Merck KGaA) was used to detect ROS levels in NRK cells according to the manufacturer's protocols. After incubating with 10 μ M DCFH-DA for 1 h at 4°C in the dark, NRK cells were treated with 1 μ g/ml DAPI for 10 min at 4°C. The cells were observed using a fluorescent microscope at a wavelength of 485 nm.

Statistical analysis. GraphPad Prism version 8.0.1 (GraphPad Software, Inc.) was used to analyze the data. Data are presented as the mean \pm SD of three repeats. Differences between groups were compared using one-way ANOVA followed by a post hoc Tukey's. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

SA protects against RIRI in a dose-dependent manner. To assess the role of SA in RIRI, rats were subjected to different doses of SA (1, 10, or 100 mg/kg/day), administered via gavage for 7 days before undergoing the establishment of RIRI. Renal function was assessed following this treatment. The results of hematoxylin and eosin staining and renal injury score analysis demonstrated reduced IR-induced renal tubular injury in the rats pretreated with SA. In the RIRI rats, pretreatment with 1, 10, and 100 mg/kg of SA significantly decreased renal tubular injury, with the optimal effect apparent at 10 and 100 mg/kg, whereas the renal protective effect of 1 mg/kg SA pretreatment was less prominent (Fig. 1A and B). Accordingly, 10 mg/kg SA was used for all subsequent experiments. Moreover, the concentrations of Scr and BUN in the IR group were notably higher than those in the sham group. Compared to the IR group, pretreatment

with SA significantly reduced Scr and BUN levels in the serum (Fig. 1C and D).

SA inhibits IR-induced oxidative stress and ferroptosis in rats. Next, the effect of SA pretreatment on the levels of ROS in the IR-induced kidneys was examined. The results showed that SA pretreatment inhibited IR-induced increases in ROS and MDA while increasing the levels of GSH, SOD1, and SOD2 (Fig. 2A-G). Numerous studies have shown that high levels of oxidative stress can induce ferroptosis (19-21). Therefore, subsequent investigations focused on the effect of SA on the ferroptosis of renal cells during IR. The results showed that SA pretreatment inhibited the decrease in the expression of GPX4 and the increase in the expression of ACSL4 induced by RIRI (Fig. 2H-J). Furthermore, the levels of LPO were detected using the lipid peroxidation probe C11-BODIPY^{581/591}. The significant increase in LPO induced by IR was prevented by treatment with SA (Fig. 2K and L). Together, the results suggested that SA pretreatment conferred its renal protective effects through anti-oxidative and anti-ferroptotic means.

SA reduces oxidative stress induced by HR injury in NRK cells. Subsequently, *in vitro* experiments were performed to evaluate the effect of SA on the human renal tubular epithelial cell line NRK. First, the toxicity of SA in NRK cells was evaluated and it was found that SA did not noticeably impact cell viability after 24 h (Fig. 3A). Additionally, we discovered that pretreatment with SA reduced HR-induced cell death. Treatment with 1-1,000 μ M SA exerted a renal protective effect, with the optimal effect evident at 100 μ M (Fig. 3B). Therefore, a concentration of 100 μ M of SA was used for further experiments. DHE staining and MDA detection kits were used to assess the effects of SA on ROS levels induced by HR. The results showed that reduced ROS levels in NRK cells were associated with increased SOD and GSH activity and decreased activity of MDA (Fig. 3C-G). In agreement with the *in vivo* experiments, the outcomes of the *in vitro* experiments showed that SA protected renal tubular epithelial cells from oxidative damage through suppression of ROS buildup.

PI3K/AKT signaling pathway is involved in the protective effects of SA on oxidative stress against HR injury. The PI3K/AKT signaling pathway is a critical player in the modulation of oxidative stress. Therefore, to investigate whether SA exerts a protective effect on HR injury by targeting the PI3K/AKT signaling pathway, a PI3K/AKT pathway inhibitor, PI3K/AKT-IN-1 (2.62 μ M), was employed. The cell viability assay demonstrated that PI3K/AKT-IN-1 partially abrogated the protective effect of SA against HR-induced NRK cell injury (Fig. 4A). Moreover, compared to the sham group, there was an increase in the phosphorylation levels of PI3K and AKT during HR injury, and SA pretreatment further augmented the phosphorylation of PI3K and AKT. However, in the presence of PI3K/AKT-IN-1, the increased phosphorylation of PI3K and AKT was suppressed (Fig. 4B-D). Additionally, PI3K/AKT-IN-1 increased MDA levels while decreasing the levels of SOD and GSH (Fig. 4E-G). Accordingly, SA exerts its antioxidant effect on NRK cells by activating the PI3K/AKT signaling pathway.

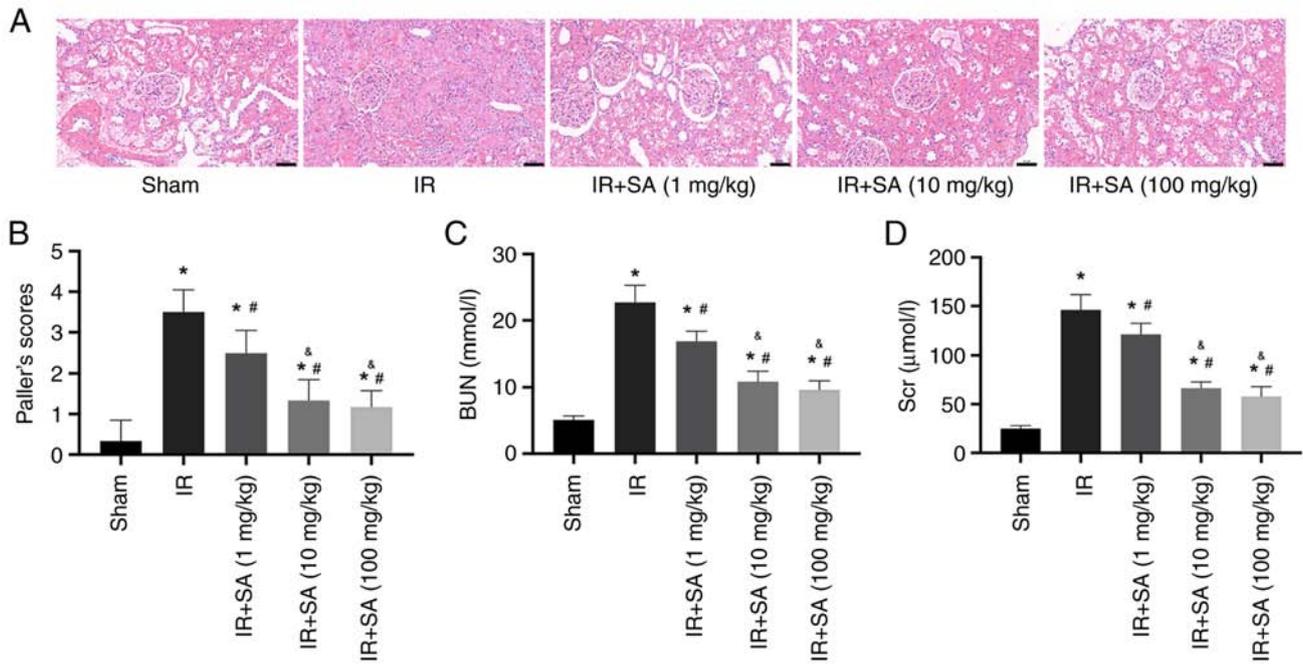


Figure 1. SA attenuates IR-induced kidney injury. Rats underwent a sham operation or renal IR with or without pretreatment with SA (1, 10, or 100 mg/kg/day). (A) Representative images of HE staining (magnification, x400) of the kidney sections. (B) Paller scores of kidney injury. (C and D) Serum concentrations of BUN and Scr in the rats. *P<0.05 vs. sham group; #P<0.05 vs. IR, &P<0.05 vs. IR+SA (1 mg/kg) group. HE, hematoxylin and eosin; BUN, blood urea nitrogen; Scr, serum creatinine; SA, Salidroside; IR, ischemia/reperfusion.

HR-induced ferroptosis can be aggravated by blocking the PI3K/AKT signaling pathway. Finally, the effects of PI3K/AKT signaling pathway inhibition on HR-induced ferroptosis of NRK cells were explored. The results of western blotting showed that PI3K/AKT-IN-1 prevented the increased expression of GPX4 and the decreased expression of ACSL4 induced by SA (Fig. 5A-C). Additionally, the levels of LPO were assessed using the lipid peroxidation probe C11-BODIPY^{581/591}. The data confirmed that PI3K/AKT-IN-1 reversed the inhibitory effect of SA on LPO (Fig. 2D and E). Collectively, the outcomes indicated that the protective effects of SA pretreatment on renal tubular epithelial cells were brought about through an anti-ferroptotic effect; however, this effect could be counteracted by inhibition of the PI3K/AKT signaling pathway.

Discussion

The results of the present study showed that SA treatment is an effective method of reducing oxidative stress and inhibiting ferroptosis during RIRI, leading to the alleviation of this condition. Therefore, the use of natural products can be considered a reference point for managing and preventing RIRI. Natural products have demonstrated good efficacy in preventing and treating AKI with minimal side effects. Among these, *Rhodiola*, a perennial herb belonging to the sedum genus in the *Sedum* family (22), is noteworthy due to the presence of SA, tyrosol, flavonoid, amino acid, trace volatile oil, and other constituents, which underlie its beneficial effects (23,24). Recent studies have demonstrated the potential of *Rhodiola rosea* to affect various biological pathways, including anti-hypoxic, anti-fatigue, anti-viral, immunity-enhancing, anti-aging, and anti-radiation pathways, and bidirectional

regulation of bodily functions (25,26). *Rhodiola* can significantly increase the endurance of hypoxia-exposed human bodies, promote aerobic metabolism, reduce the concentration of lactic acid in the blood, muscles, and brain, increase the aerobic metabolism of the heart, brain, lung, and other critical organs, assist the body in adapting to an hypoxic environment, and deferring the onset of further fatigue (27-29). SA, the primary constituent of *Rhodiola*, has been found by previous studies to safeguard organs from multiple IRI-related pathway dysfunctions (30-32).

The process of IRI involves various factors and mechanisms, including reduced nitric oxide production, inflammation-induced cell damage, oxygen-derived oxidative stress formation, and lipid peroxidation (33). Prior work has identified oxidative stress as a key potential mechanism of tissue damage induced by IRI (34). Oxidative-free radicals and ROS scavengers are vital for maintaining the oxidant-anti-oxidant balance *in vivo* during IRI (35). Excessive generation of oxygen-free radicals by ischemia-reperfusion can surpass local tissue scavenging capacity and lead to damage (36). MDA serves as a stable metabolic intermediate indicative of lipid peroxidation levels and oxygen-free radical content in tissues. Prior research has shown that IRI elevates ROS and MDA levels (35). The present study also confirmed that RIRI led to a significant increase in oxidative stress and that pretreatment with SA mitigated this, demonstrating the protective effect of SA by inhibiting oxidative stress and ferroptosis.

SA is a widely studied natural antioxidant with potent antioxidant effects. AKT, which is mediated by phosphoinositol-dependent kinase-1 (PDK-1) (37), acts as a downstream factor of PI3K. Studies have suggested that natural antioxidants can activate PI3K by binding to GPCRs and EGFR (38,39). Therefore, it was hypothesized

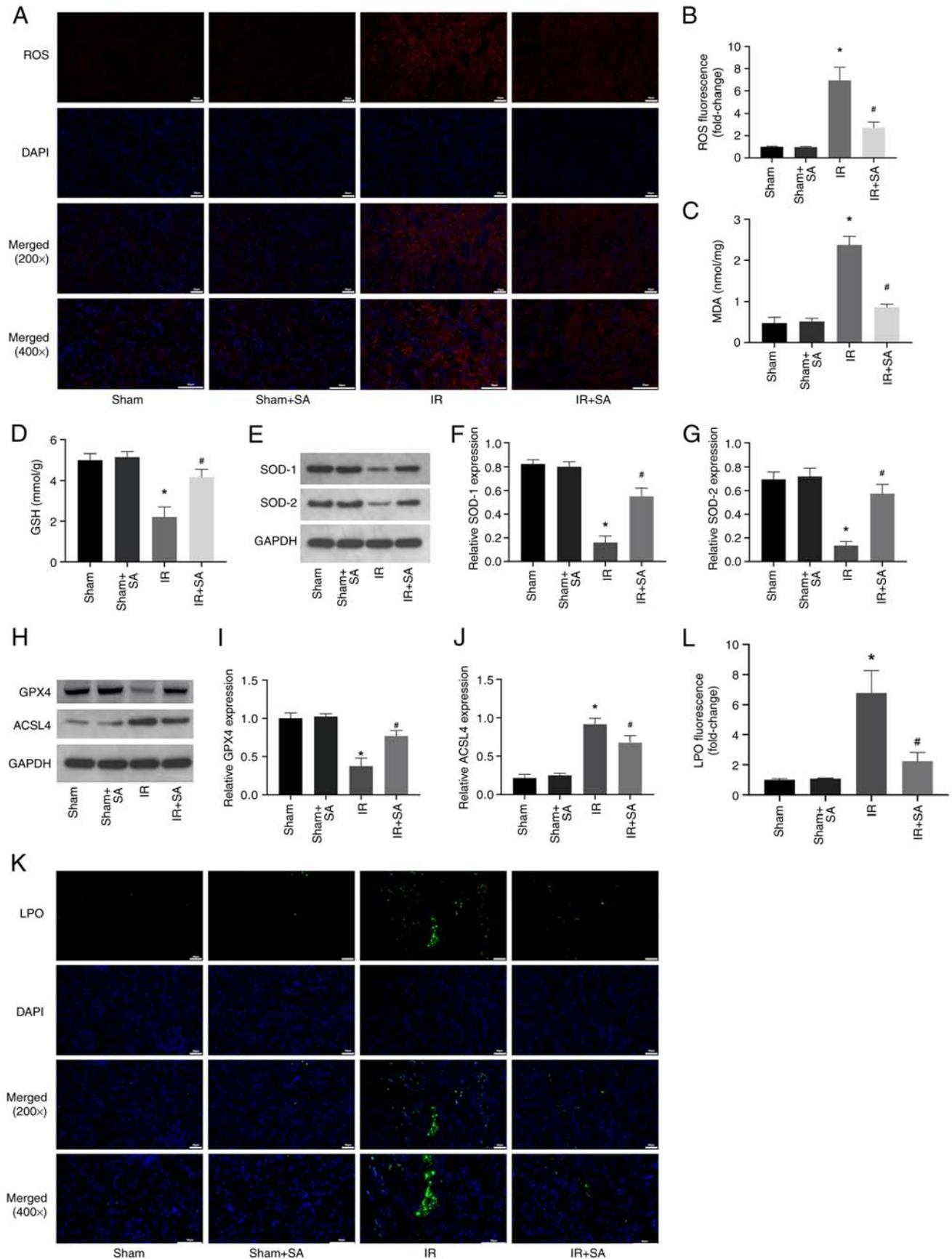


Figure 2. SA inhibits IR-induced increases in oxidative stress and ferroptosis in rats. (A and B) Representative images of kidney sections stained with dihydroethidium and semi-quantitative analysis of fluorescence intensity indicated that SA pretreatment reduced the ROS levels in rats subjected to RIRI (magnification, x200 and x400). (C and D) The levels of MDA and GSH in kidney tissues were detected. (E-G) SOD1 and SOD2 levels were detected using western blotting. (H-J) The expression of GPX4 and ACSL4 was detected using western blotting. (K and L) The levels of LPO were detected using the lipid peroxidation probe C11-BODIPY^{581/591} (magnification, x200 and x400). *P<0.05 vs. sham group, #P<0.05 vs. IR. SA, Salidroside; IR, ischemia/reperfusion; ROS, reactive oxygen species; RIRI, renal ischemia/reperfusion injury.

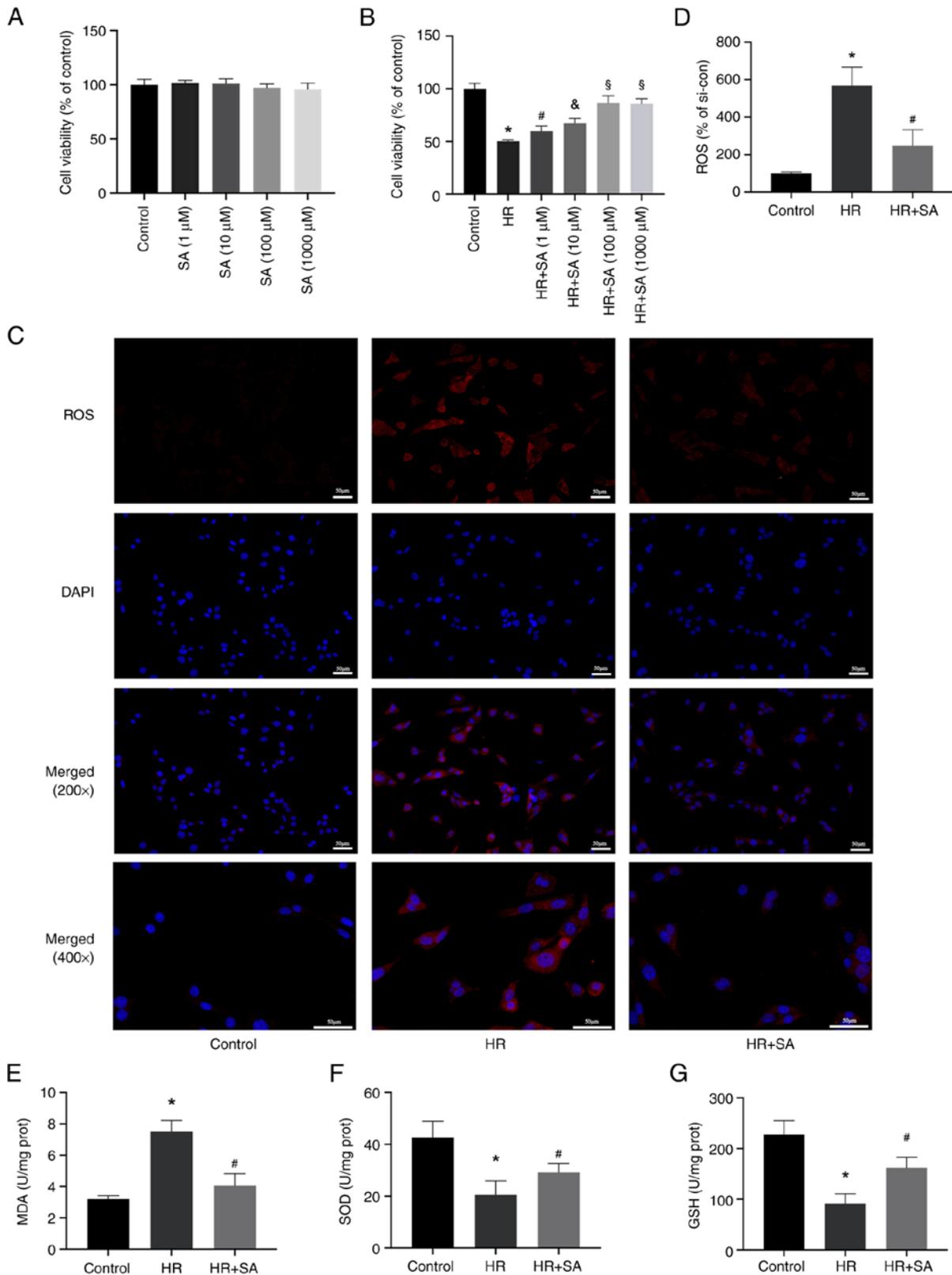


Figure 3. SA reduces oxidative stress induced by HR injury in NRK cells. (A) SA had no notable toxic effects on NRK cell viability. (B) SA (1-1,000 μ M) had notable renal-protective effects on cell viability. (C and D) Representative images and statistical analysis of dihydroethidium staining indicated that SA pretreatment reduced ROS content in HR-induced NRK cells (magnification, x200 and x400). (E-G) MDA levels were reduced while SOD and GSH activity increased in cells pretreated with SA in HR-induced NRK cells. * $P < 0.05$ vs. control group; # $P < 0.05$ vs. HR group; & $P < 0.05$ vs. HR+SA (1 μ M) group; § $P < 0.05$ vs HR+SA (10 μ M) group. SA, Salidroside; HR, hypoxia-reoxygenation.

that SA, like other natural antioxidants, may activate the PI3K/AKT signaling pathway; prior work confirmed that SA

indeed activates this pathway (11,40). In the present study, SOD1/2 expression and activity in IRI kidneys were initially

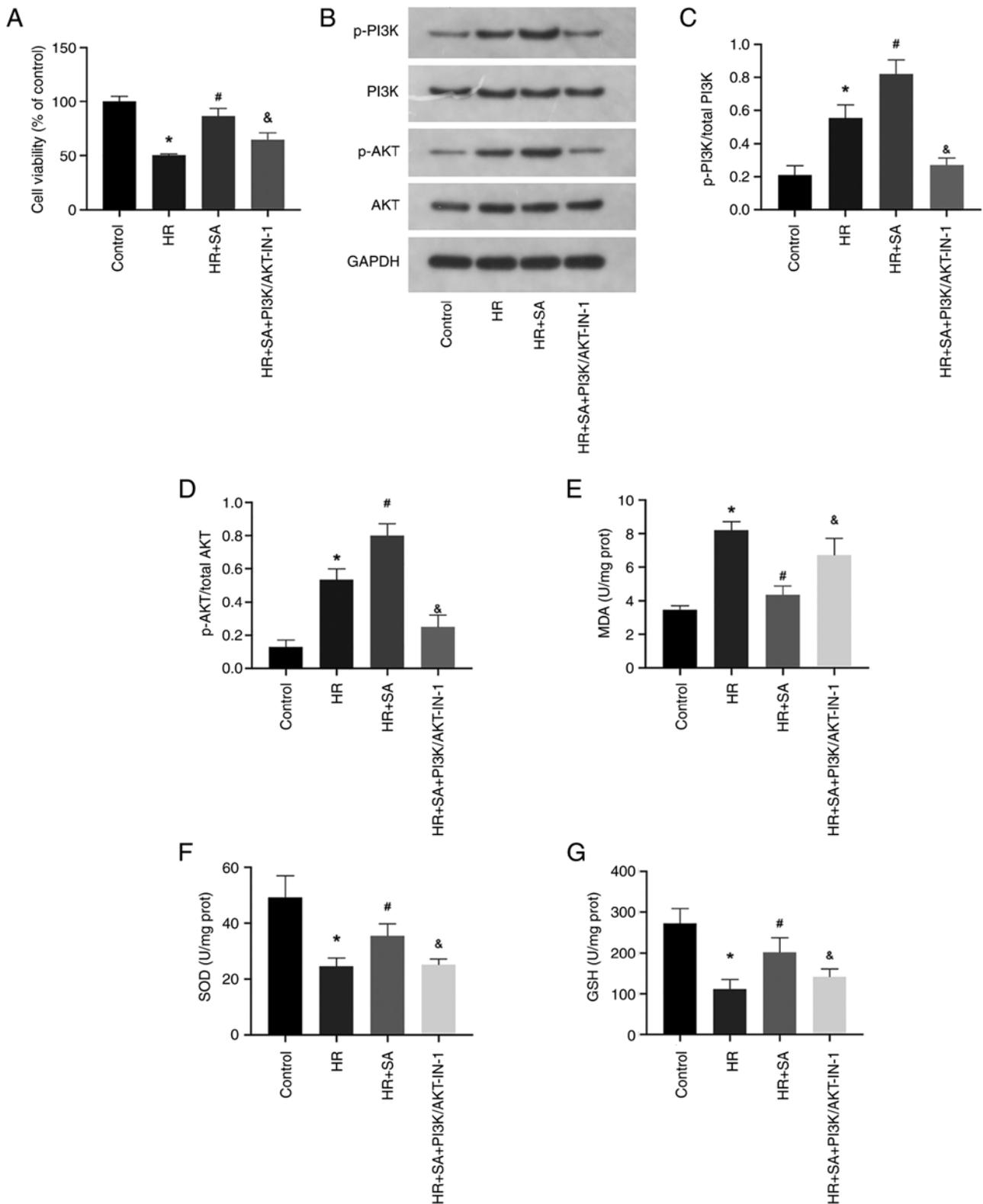


Figure 4. The PI3K/AKT signaling pathway is involved in the protective effects of SA on oxidative stress against HR injury. (A) PI3K/AKT-IN-1 partially reversed the protective effects of SA on HR-induced NRK cell injury. (B-D) HR-induced PI3K and AKT phosphorylation increased with SA pretreatment but was blocked by PI3K/AKT-IN-1. (E-G) Levels of MDA, SOD, and GSH were detected. Magnification, x200 and x400. *P<0.05 vs. control; #P<0.05 vs. HR group; &P<0.05 vs. SA+HR. SA, Salidroside; HR, hypoxia-reoxygenation.

evaluated, followed by verification of these findings using NRK cells. SA pretreatment increased SOD activity by further activating the PI3K/AKT signaling pathway during HR treatment. These results confirmed that the PI3K/AKT

signaling pathway underlies the anti-oxidative stress effect of SA.

Ferroptosis is a form of cell death resulting from lethal lipid peroxidation and iron-dependent damage of membrane

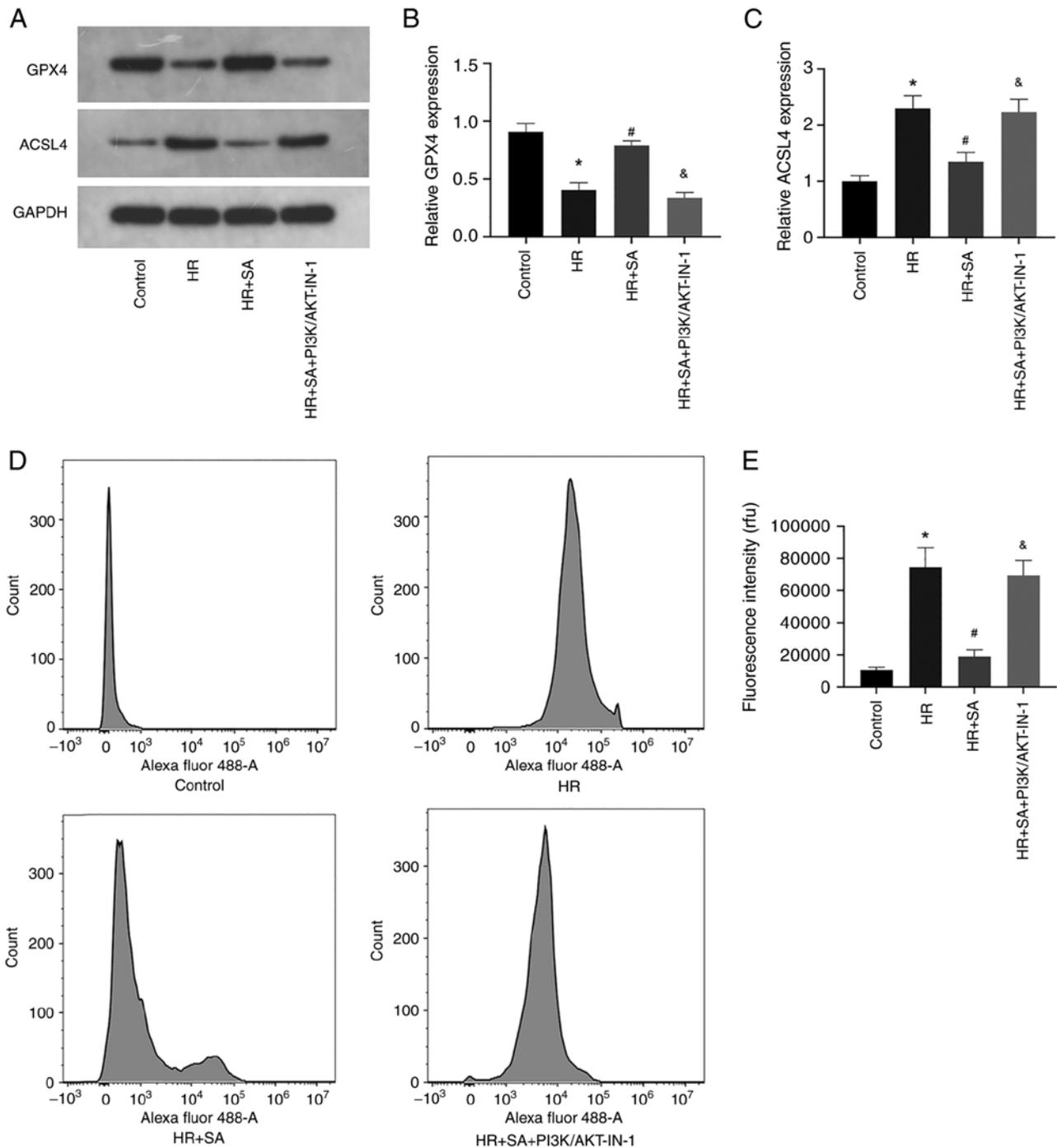


Figure 5. HR-induced ferroptosis is aggravated by blocking the PI3K/AKT signaling pathway. (A-C) The expression of GPX4 and ACSL4 was detected using western blotting. (D and E) The levels of LPO were detected using flow cytometry. * $P < 0.05$ vs. control group; # $P < 0.05$ vs. HR group; & $P < 0.05$ vs. SA+HR. SA, Salidroside; HR, hypoxia-reoxygenation.

lipids. Ferroptosis is involved in various organ injuries induced by IRI, including the heart, liver, kidney, and brain (41-44). Nonetheless, it remains unclear whether SA can exert a protective effect by inhibiting ferroptosis during RIR. Inactivation of the lipid repair enzyme GPX4 leads to the accumulation of lipid peroxides, including lipid hydrogen peroxide, as GPX4 is the sole enzyme capable of reducing lipid peroxidation in biofilms, and GSH is a necessary co-factor for GPX4 enzyme activity. GPX4 inactivation results in the accumulation of high levels of ROS, ultimately leading to cell damage. The results of the present study revealed that SA enhanced the antioxidant

capacity of the kidney by activating the PI3K/AKT signaling pathway, thereby inhibiting ferroptosis induced by high levels of oxidative stress. In short, SA exhibits a potent anti-ferroptotic effect on NRK cells, and this effect can be reversed by inhibiting PI3K/AKT. Consequently, ferroptosis induced by ROS is also suppressed. Additionally, treatment with the PI3K/AKT pathway inhibitor, PI3K/AKT-IN-1, inhibited the activation of the PI3K/AKT pathway and its downstream regulation of oxidative stress and ferroptosis. These findings support the notion that SA pretreatment can alleviate RIRI by activating the PI3K/AKT signaling pathway.

However, the present study has some limitations. First, SA may be used as a clinical agent to treat RIRI, thus the efficacy of SA in patients who already have RIRI should be explored. Secondly, it was confirmed that 10-100 mg/kg SA had a strong renal protective effect; however, the optimal dose of SA was determined. These factors should be considered in further studies.

In conclusion, the present study showed that SA increased the activity of SODs by activating the PI3K/AKT signaling pathway, thereby eliminating ROS, and inhibiting oxidative stress injury and ferroptosis, thus protecting renal function in RIRI.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

ZT and CL conceived and designed the study, analyzed the data, and wrote the manuscript. YW collected and analyzed the data. YL analyzed the data and wrote the manuscript. CL and ZT confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was performed in accordance with the Helsinki Declaration II and was approved by the Institutional Review Boards of Renmin Hospital of Wuhan University (approval no. K2021-08-012).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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