Luteolin alleviates sorafenib-induced ferroptosis of BRL-3A cells through modulation of the Nrf2/GPX4 signaling pathway

Bo-Wen Zhang¹,², Di Yang¹,³, Jin-Tao Li¹,³, Mei-Hao Peng¹,³, Jia-Qing Liao¹,³, Qi Zhao¹,³, Yi-Xi Yang¹,², Qiu-Xia Lu¹,²*¹

¹Engineering Research Center of Sichuan-Tibet Traditional Medicinal Plant, Chengdu University, Chengdu 610106, China. ²School of Food and Biological Engineering, Chengdu University, Chengdu 610106, China. ³School of Pharmacy, Chengdu University, Chengdu 610106, China.

Correspondence to: Yi-Xi Yang, Qiu-Xia Lu. Engineering Research Center of Sichuan-Tibet Traditional Medicinal Plant, Chengdu University, No. 2025, Shiling Street, Chengdu Avenue, Chengdu 610106, China. E-mail: yangyx1011@cdtu.edu.cn; luqixia@cdtu.edu.cn.

Author contributions
Zhang BW and Yang D performed the experiments and analyzed the data; Zhang BW, Yang D, Li JT, Peng MH, Liu XJ, Yang YX, and Lu QX wrote the manuscript; Zhao Q, Yang YX, and Lu QX all contributed to the conception and design of this study, manuscript revision, project management, and project funding. All data were generated inhouse, and no paper mill was used. All authors have read and agreed to the published version of the manuscript.

Competing interests
The authors declare no conflicts of interest.

Acknowledgments
This study was supported by the open fund of State Key Laboratory of Southwestern Chinese Medicine Resources (No. SCMR202103) to Jian Li, Tibet Autonomous Region Science and Technology Plan (high-tech social development) project (No. ZX2020Z010031G) to Yang YX and Anti-infective Agent Creation Engineering Research Centre of Sichuan Province, Sichuan Industrial Institute of Antibiotics, School of pharmacy, Chengdu University (No. AAC2023002) to Lu QX.

Peer review information
Traditional Medicine Research thanks all anonymous reviewers for their contribution to the peer review of this paper.

Abbreviations
BSA, bovine serum albumin; CCK8, cell counting kit-8; FER-1, ferrostatin-1; GPX4, glutathione peroxidase 4; GSH, glutathione; HO-1, heme oxygenase 1; MDA, malondialdehyde; NQO1, quinone oxidoreductase 1; IC-1, 5,5',6,6'-Tetrachloro-1,1,3,3-tetraethyl-imidacarbocyanine iodide; Nrf2, nuclear factor E2-related factor 2; SD, standard deviation; ns, no significance; PBS, phosphate-buffered saline; ROS, reactive oxygen species; RT-qPCR, real-time quantitative PCR; SLC7A11, cystine/glutamate transporter xCT.

Citation

Abstract
Background: Luteolin is a flavonoid chemical that exists in a variety of medicinal and edible plants and holds many biologically active properties in liver protection, anti-cancer, antioxidants, anti-inflammatory, neuroprotective, etc. According to its hepatoprotective properties, luteolin was selected to co-treat with sorafenib, one of the approved protein kinase inhibitors, to reduce sorafenib-induced normal liver cell damage. Methods: The BRL-3A cell line was treated with sorafenib to establish a liver injury model, followed by luteolin treatment. The cell viability was detected, and the mechanism of action was detected by immunofluorescence, western blotting, and real-time quantitative PCR. Results: The research findings demonstrated that luteolin could increase cystine/glutamate transporter xCT (SLC7A11) and glutathione peroxidase 4 (GPX4) expression and display a chelating effect on iron, which led to increased glutathione and decreased malondialdehyde, Fe²⁺ and lipid reactive oxygen species contents in BRL-3A cells, and the sorafenib-induced mitochondrial membrane potential decrease was also inhibited. In addition, when sorafenib caused the accumulation of lipid reactive oxygen species, luteolin could help release this oxidative stress by activating nuclear factor E2-related factor 2 (Nrf2) and up-regulating the expression of the associated genes heme oxygenase 1 (HO-1) and quinone oxidoreductase 1 (NQO1). Conclusion: Therefore, luteolin may ameliorate sorafenib-induced ferroptosis by activating the Nrf2-associated pathway without any impact on sorafenib anti-cancer activity. It can be used as an adjuvant to sorafenib to reduce liver injury in patients with hepatocellular carcinoma.

Keywords: luteolin; sorafenib; liver injury; ferroptosis; Nrf2/GPX4
Sorafenib-induced damage to healthy liver cells.
Consequently, the purpose of this article is to clarify whether sorafenib induces the death of normal hepatocytes in terms of ferroptosis and oxidative stress. What’s more, the molecular mechanism of luteolin’s protective impact against sorafenib-induced hepatotoxicity needs to be investigated.

Methods
Cell culture and reagents
The BRL-3A and HepG2 (Cell Bank of the Chinese Academy of Sciences in Shanghai, China) were cultured in Dulbecco’s modified eagle medium (Wisent, St-Bruno, Canada) with 10% fetal bovine serum (Wisent, St-Bruno, Canada), and put in a cell growth incubator at 37°C with 5% CO₂.
Luteolin (Selleck, Shanghai, China, Purity: 99.04%), sorafenib (Selleck, Shanghai, China, Purity: 99.88%), erastin (Topscience, Shanghai, China, Purity: 99.04%) and ferrostatin-1 (Velas, Topscience, Shanghai, China, Purity: 99.96%) were dissolved in dimethyl sulfoxide (DMSO, Wisent, St-Bruno, Canada) at 180 μM, 25 mM, 5 mM and 1 mg/mL concentrations as stock solutions.

The treatment of BRL-3A and HepG2 cells
BRL-3A and HepG2 cells were cultured in 6-well, 24-well, or 96-well plates and treated with different concentrations of luteolin and sorafenib combination for 12 h. This method was used for subsequent activity, lipid ROS, malondialdehyde (MDA), glutathione (GSH), Fe²⁺ level determination, mitochondrial membrane potential, mitochondrial lipid ROS, qPCR, western blotting, and immunofluorescence.

Cell viability assays
BRL-3A and HepG2 cell density was adjusted to 1.5 × 10⁴ cells/mL before inoculating the 96-well plate using. The control group, model group, different concentrations of luteolin groups, and positive control Fer-1 group were set up. After 12 h, each well was incubated with 10% cell counting kit-8 (CCK8, Beyotime, Shanghai, China) medium for 1 h. The absorbance was measured at 450 nm under the microplate reader.

Lipid ROS level determination
BRL-3A cells (8 × 10⁴ cells/mL) were inoculated in the 24-well plates. After 12 h of treatment with the methods described above, and the amount of the C11 dye (Thermofisher, Shanghai, China) working solution was added, the cells were cultured for 30 min at 37°C with 5% CO₂, and cleaned 3 times with phosphate-buffered saline (PBS, Wisent, St-Bruno, Canada). The next observation was taken by the fluorescent microscope (Nikon, Tokyo, Japan) after 200 μL of blocking dye was added.

MDA and GSH and Fe²⁺ level determinations
Trypsin 500 μL was used to digest the cells in the 6-well plates, which were transferred to EP tubes, washed twice with PBS at 1,000 rpm for 3 min, and then 400 μL PBS was added to keep the cells suspended. The cells were sonicated 5 times with 20%–30% power of 150 W, 5 s/time and 30 s interval. The levels of MDA (A003-1, Nanjing Jiancheng, Nanjing, China), GSH (A006-2-1, Nanjing Jiancheng, Nanjing, China) and Fe²⁺ (A039-1, Nanjing Jiancheng, Nanjing, China) were determined using the kit instructions.

Mitochondrial membrane potential level determination
The working solution of 5,5’,6,6’-Tetrachloro-1,1’,3,3’-tetraethyl-imidacarbocyanine iodide (JC-1) was prepared following the JC-1 assay kit (Beyotime, Chongqing, China) instruction. Medium containing 10% fetal bovine serum was washed with PBS. When mixed fully with 480 μL of working solution of JC-1, the cells were cultured for 40 min at 37°C with a 5% CO₂ incubator away from light. Then the cells were twice rinsed with JC-1 buffer, and 2 mL of culture medium was added for the image by fluorescence microscopy.
Figure 1 Effect of luteolin pretreatment on sorafenib-induced hepatotoxicity in BRL-3A and HepG2 cells. (A) The chemical structure of luteolin. (B) BRL-3A cells were treated by sorafenib (0–100 μM) for 12 h. The cell viability was measured by the CCK8 assay (n = 3). (C) HepG2 cells were treated by sorafenib (0–100 μM) for 24 h. The cell viability was measured by the CCK8 assay (n = 3). (D) BRL-3A cells were treated by luteolin (0.008–50 μM) for 24 h. The cell viability was measured by the CCK8 assay (n = 3). (E) HepG2 cells were treated by luteolin (0.008–50 μM) for 24 h. The cell viability was measured by the CCK8 assay (n = 3). (F) The survival rate of HepG2 cells after co-treatment with luteolin (0.2–5 μM) and sorafenib (25 μM) for 12 h (n = 3). (G) The survival rate of BRL-3A cells after co-treatment with luteolin (0.2–5 μM) and sorafenib (25 μM) for 12 h (n = 3). (H) Protection of luteolin or Fer-1 on sorafenib in morphological changes under a microscope (n = 3). Plotting scale is 100 μM. All results were expressed as means ± standard deviation (SD) of three independent experiments. Compared to the control group: ****P < 0.0001; compared to the model group: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. ns, no significance; Fer-1, ferrostatin-1.

Mitochondrial lipid ROS level determination
The reagents were prepared following the mtROS test kit (Dojindo, Shanghai, China) instructions. Cells were washed with PBS twice and then MitoPeDPP working solution (Dojindo, Shanghai, China) was added and the cells were left to be incubated at 37 °C for 15 min. Then, HEPE buffer (Macklin, Shanghai, China) was added, and the cells were visualized using a fluorescent microscope.

Real-time quantitative PCR (RT-qPCR) assay
Total cellular RNA was extracted using a Trizol kit (Vazyme, Nanjing, China), and then reverse transcription was performed with the cDNA synthesis kit (Vazyme, Nanjing, China). The RT-qPCR was performed using a real-time quantitative fluorescence PCR instrument and software. The relative expression of the mRNA of quinone oxidoreductase 1 (NQO1), HO-1, GPX4, and cystine/glutamate transporter xCT (SLC7A11) was calculated by using β-actin as an internal control with the 2^{-ΔΔCt} technique. Each sample was tested at least three times independently. The primer sequences are displayed in the Supplementary Table S1.

Western blotting
The RIPA lysate, in a volume of 80 μL per well, was used to extract total protein. Electrophoresed at 80 V for 90 min and wet-turned at 200 mA for 120 min. After blocking with 5% non-fat milk at ambient temperature for 1 h, the membranes with different primary antibodies, which were Nrf2 (1:1,000, 127217, Cell Signaling Technology, Waltham, MA, USA), NQO1 (1:1,000, ER1802-85, Huabio, Hangzhou China), HO-1 (1:1,000, 67763-1-1g, Proteintech, Wuhan, China), GPX4 (1:1,000, 67763-1-1g, Proteintech, Wuhan, China), SLC7A11 (1:1000, PA1-16893, Thermo Fisher Scientific, Waltham, MA, USA) and β-actin (1:1,000, 680008-1-lg, Proteintech, Wuhan, China) respectively, were incubated overnight at 4 °C. Afterward, the membranes were incubated with the secondary antibodies (anti-mouse (HA1006, Huabio, Hangzhou China) and anti-rabbit (HA1001, Huabio, Hangzhou China)) for 1 h at ambient temperature. The signal was detected by an enhanced chemiluminescence reagent (Thermo Fisher Scientific, Waltham, MA, USA), and the grayscale analysis was performed using ImageJ software.

Nrf2 immunofluorescence assay
Cells were fastened in 500 μL of 4% paraformaldehyde for 15 min. The cells were washed with PBS three times for 5 min and subsequently permeabilized in 400 μL of 0.1% Triton for 15 min. After being washed by PBS three times for 5 min each again, 500 μL of 1% bovine serum albumin (BSA) was used to block cells for 1 h. Afterward, cells were incubated using Nrf2 antibody (1:1,000) with 1% BSA overnight at 4 °C and rinsed with PBS 3 times. Then, cells, mixed with the secondary antibody (1:1,000) with 1% BSA, were incubated for 1 h at ambient temperature and wrapped in tin foil. After being rinsed with PBS three times again, the nucleus was stained using 200 μL of anti-fluorescence quencher containing 4′,6-diamidino-2-phenylindole.
(DAPI), and cells were observed by a fluorescence microscope.

Statistical analysis
Excel and GraphPad Prism 7.0 were used to analyze the data statistically. The measurement data were given in the form of mean ± SD. One-way ANOVA was employed, and $P < 0.05$ was declared of statistical significance.

Results
Luteolin reduces sorafenib-induced BRL-3A cell death
Sorafenib was used to induce BRL-3A cell injury to construct a liver damage model. Following a 12 h treatment with different doses of sorafenib (Figure 1B, 1C). The modeling concentration of sorafenib was chosen to be 25 μM for subsequent experiments based on the fact that cell survival rate decreased to 50% when sorafenib was administrated at this dosage. Then, several amounts of luteolin were applied to the BRL-3A cells and HepG2 cells (50, 25, 5, 1, 0.2, 0.04 and 0.008 μM) for 24 h (Figure 1D, 1E), where there were no significant differences between the control group and luteolin groups in the concentration range from 50 to 0.008 μM on cell survival rate.

Moreover, luteolin and sorafenib were co-treated on HepG2 cells and BRL-3A cells for 12 h (Figure 1F, 1G), all these findings proved that luteolin and Fer-1 could alleviate sorafenib-induced hepatocyte injury without influence the anti-cancer activity of sorafenib. The observation of the microstructure (Figure 1H) further illustrated this phenomenon.

Luteolin alleviates sorafenib-induced ferroptosis
A fluorescent probe was used to measure intracellular lipid ROS levels, the accumulation of which directly leads to the new programmed cell death called ferroptosis [13]. C11 dye was used, which could assemble in the membrane and bind to lipid ROS to emit green fluorescence. The higher the amount of lipid ROS, the brighter the green fluorescence [18]. The model group had the brightest green fluorescence (Figure 2A). This is because sorafenib could increase oxidative stress, which could lead to more lipid ROS. When luteolin was combined with sorafenib, sorafenib-induced lipid ROS formation was dramatically reduced, and only a few green fluorescence was observed by the fluorescent microscope, which were similar to those in the positive control group.

MDA content, one of the indicators for lipid peroxidation, is the product of the last stage of lipid oxidation [19]. In this study, the MDA content of the cells was highest in the model group, and it was decreased by the co-treatment of luteolin at the concentrations of 1 and 5 μM. In addition, the MDA content of the luteolin group at a concentration of 1 μM was almost identical to that of the positive control group, and both groups didn’t show any significant difference (Figure 2B). GSH and Fe$^{2+}$ contents are two key ferroptosis indicators from two different pathways. Sorafenib could inhibit the expression of SLC7A11 and hinder the synthesis of GSH. Meanwhile, sorafenib also leads to Fe$^{2+}$ overload and causes damage to mitochondrial function. Both intracellular GSH and mitochondrial function damage cause the accumulation of lipid ROS. When co-treatment with luteolin, it was found that GSH content increased compared with the model group, while its Fe$^{2+}$ content sharply dropped (Figure 2C, 2D). The above results suggested that luteolin can effectively alleviate sorafenib-induced ferroptosis by reducing lipid peroxidation and iron chelation.

Erastin validates that luteolin alleviates ferroptosis.
Erastin, an internationally recognized inducer of ferroptosis, was utilized instead of sorafenib to demonstrate that luteolin really is related to the inhibition of ferroptosis [20]. Where erastin dramatically decreased BRL-3A cell viability, however, luteolin and Fer-1 recovered cell viability in a dosage-dependent pattern (Figure 3A). Furthermore, the status of lipid ROS for each group was assessed using a fluorescent probe (Figure 3B). It can be observed under fluorescent microscopy that lipid ROS accumulated in the erastin-treated cells, which emitted bright green fluorescence, but both luteolin and Fer-1 groups didn’t observe these signals, which proved luteolin had the opposite ability of erastin to reduce the lipid ROS production and inhibit the ferroptosis.

Effect of luteolin on mitochondrial damage induced by sorafenib in BRL-3A cells
When mitochondria are healthy and have a higher membrane potential, JC-1 assemblies in the mitochondrial matrix as a polymer that fluoresces red; when the mitochondrial membrane potential is decreased or disappears, JC-1 presents as a monomer in the cytoplasm and emits green fluorescence [21]. When cells were treated with sorafenib, the ratio of red to green fluorescence decreased. This suggests that the damage to mitochondrial function led to a drop in the potential of the mitochondrial membrane (Figure 4A). After the co-treatment with luteolin, the ratio of red to green fluorescence clearly increased with concentration, which indicated luteolin restored mitochondrial function. The ability of Fer-1 at 1 μM only reached that of luteolin at 0.2 μM.

Figure 2 Luteolin reduced sorafenib-induced oxidative stress in BRL-3A cells. (A) The effect of luteolin against sorafenib-induced lipid ROS production was observed by fluorescence microscopy and quantification plots (n = 3). Plotting scale = 20 μM. (B-D) Effect of luteolin on MDA, GSH and Fe$^{2+}$ contents. All results were expressed as means ± SD of 3 independent experiments (n = 3). Compared to the control group: $^{\ast\ast}P < 0.01$, $^{\ast\ast\ast}P < 0.0001$; compared to the model group: $^{\ast\ast}\star P < 0.001$, $^{\ast\ast\ast}\star P < 0.0001$. Fer-1, ferrostatin-1; ns, no significance; ROS, reactive oxygen species; MDA, malondialdehyde; GSH, glutathione.
Figure 3 Erastin verifies that luteolin alleviates ferroptosis in BRL-3A cells. (A) The survival rate of BRL-3A cells after co-treatment with luteolin (0.2–5 μM) and erastin (2 μM) for 12 h (n = 3). (B) The effect of luteolin against erastin-induced lipid ROS production was observed by fluorescence microscopy (n = 3). Plotting scale = 20 μM. All results were expressed as means ± SD of three independent experiments. Compared to the control group: """"P < 0.0001; compared to the model group: """"""""P < 0.0001. Fer-1, ferrostatin-1; ROS, reactive oxygen species.

Figure 4 Luteolin inhibits sorafenib-induced mitochondrial membrane potential decrease and lipid ROS increase in BRL-3A cells. (A) Changes in mitochondrial membrane potential level and quantification plots (n = 3). Plotting scale = 50 μM. (B) Changes in mitochondrial lipid ROS level and quantification plots (n = 3). Plotting scale = 20 μM. Compared to the control group: """"""""P < 0.0001; compared to the model group: """"""""P < 0.0001. Fer-1, ferrostatin-1; ROS, reactive oxygen species.

Because of the disorder of mitochondrial function, lipid ROS levels may also increase. It's indicated that bright green fluorescence was shown in the model group, but few can be found in the luteolin groups and the positive control Fer-1 group (Figure 4B). All of these suggested that luteolin could reduce the sorafenib-induced mitochondrial damage.

Luteolin relieves sorafenib-induced ferroptosis in BRL-3A cells by activating the Nrf2/GPX4 signaling pathway

To determine the specific mechanism of luteolin in regulating ferroptosis, the impacts of luteolin on SLC7A11, GPX4, HO-1, NQO1, and Nrf2 expressions were studied. While western blot and RT-qPCR were examined (Figure 5A–5I), it showed that compared with the control group, sorafenib indeed decreased the expressions of SLC7A11 and GPX4 proteins, and the GPX4 protein relative level almost decreased by 80%. After the administration of luteolin, the expressions of GPX4 and SLC7A11, similar to those in the positive control group, were slightly increased. Furthermore, the expression of
SLC7A11 mRNA in the model group was higher than that in the control group due to different detection methods, but the expression of SLC7A11 mRNA was up-regulated by luteolin, especially the high dose of luteolin. Additionally, sorafenib cannot activate the expression of HO-1, NQO1, and total Nrf2 from the Nrf2 pathway. However, luteolin treatment up-regulated these proteins and the mRNA expression levels of HO-1 and NQO1.

It is postulated that aberrant Nrf2 signaling may contribute to illnesses associated with lipid peroxidation and ferroptosis. Luteolin treatment caused the transliteration of Nrf2 to move to the nucleus and facilitated HO-1 and NQO1 transcription. According to the findings (Figure 5J), Nrf2 was distributed in the cytoplasm in the model group, while Nrf2 was distributed in and around the nucleus in the luteolin and positive control groups. These immunofluorescence results showed that sorafenib reduced FITC fluorescence intensity in the nucleus, but luteolin could keep Nrf2 expression stable in the nucleus. It’s supposed that the translocation of Nrf2 induced by luteolin treatment to the nucleus facilitated HO-1 and NQO1 transcription.

Figure 5 Ferroptosis pathway expression of related proteins and mRNAs and Nrf2 immunofluorescence analysis. (A–F) Western blotting to detect the Nrf2, HO-1, NQO1, SLC7A11, GPX4 and β-actin protein expression levels in the Nrf2/GPX4 pathway (n = 3). (G–I) RT-qPCR to detect NQO1, HO-1, SLC7A11, and β-actin mRNA expression levels (n = 3). (J) Nrf2 immunofluorescence analysis (n = 3). Plotting scale = 20 μM. Compared to the control group: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; compared to the model group: *P < 0.05, **P < 0.01, ***P < 0.001. Fer-1, ferrostatin-1; Nrf2, nuclear factor E2-related factor 2; HO-1, heme oxygenase 1; NQO1, quinone oxidoreductase 1; SLC7A11, cystine/glutamate transporter xCT; GPX4, glutathione peroxidase 4; ns, no significance.

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Discussion

Sorafenib is now the most effective first-line treatment for individuals with advanced hepatocellular carcinoma [22]. However, recent reports have connected sorafenib to hepatotoxicity, such as hepatitis, and abnormal liver function [23]. In this study, we discovered that sorafenib could induce damage to normal BRL-3A cells and cause cell shrinkage. Therefore, an effective new compound needs to be discovered to recover from this adverse reaction. Luteolin is a kind of flavonoid that has been discovered in a range of edible and medicinal plants and has the potential to protect the liver [24]. However, few studies have looked into whether luteolin can prevent liver damage from sorafenib. In this study, it’s found that luteolin could restore the normal morphology of the BRL-3A cells and increase cell vitality, indicating that luteolin could alleviate sorafenib-induced damage in BRL-3A cells. Ferroptosis belongs to a ferrous-dependent form of lipid peroxide-driven dying of cells with two characteristics: the buildup of unstable, reactive iron ions and the overload of lipid peroxides [13]. In pathological circumstances, Fe⁺⁺ penetrates the cell and transforms into Fe⁺, which then builds up inside the cell, conducts the Fenton reaction, creates lipid peroxides, damages the structure of the cell membrane, and results in cellular ferroptosis [25]. The findings revealed that both erastin and sorafenib caused the accumulation of lipid ROS in BRL-3A cells as well as a rise in MDA, the end product of lipid oxidation. Additionally, sorafenib can cause an increase in Fe⁺⁺. These results suggest that sorafenib can cause ferroptosis in BRL-3A cells. Fer-1 has been reported to be a potent and selective inhibitor of ferroptosis by iron chelation and restrain of lipid peroxides generation inhibition ferroptosis [26]. Additionally, the contents of lipid ROS, MDA, and Fe⁺⁺ were reduced by Fer-1 and luteolin, and the cell damage brought on by erastin and sorafenib was decreased. This suggests that luteolin can inhibit ferroptosis through iron chelation and restrain lipid peroxides.

System xc- is a significant isofrom of the amino acid transporter family, and its upper light chain, SLC7A11, is mainly highly specialized for cystine and GSH [27]. Studies have shown that inhibition of the expression of SLC7A11 could block the production of GSH, which reduces downstream target gene GPX4 expression [28]. Therefore, the function of SLC7A11 is essential for maintaining cellular health and survival. When the expressions of ferroptosis-related proteins GPX4 and SLC7A11 were inhibited by sorafenib, which promoted the decrease of GSH and the increase of Fe⁺⁺, then the lipid ROS accumulated and finally cause the occurrence of ferroptosis. However, luteolin and Fer-1 may reverse this tendency.

Fang et al. reported that mitochondria play a major role in the regulation of ferroptosis [29]. Mitochondrial phosphorylation by oxidation is the primary generator of ROS. When there is an excessive quantity of Fe⁺⁺, ROS in the mitochondrial membrane compartment can induce lipid peroxide production, thereby impairing mitochondrial structure and function [30]. Sorafenib reduced mitochondrial membrane potential while increasing mitochondrial lipid ROS in BRL-3A cells. It is possible that luteolin can improve mitochondrial functional impairment because luteolin and Fer-1 raise mitochondrial membrane potential and decrease the buildup of mitochondrial lipid ROS.

On the other hand, Nrf2 is an important cytoprotective factor that can be activated in cells to initiate a series of antioxidant reactions. Nrf2 can promote the expression of a series of antioxidant genes, such as HO-1 and NQO1, thereby scavenging lipid ROS, combating the occurrence of ferroptosis, and lessening oxidative injury in hepatocytes [31]. In this study, sorafenib inhibited the Nrf2 activation and down-regulated the expression of the associated genes HO-1 and NQO1. Luteolin treatment caused the trans situation of Nrf2 to move to the nucleus and facilitate HO-1 and NQO1 transcription. Thus, especially in the face of oxidative stress, luteolin fully displayed the function of a ferroptosis inhibitor and finally decreased the content of lipid ROS (Figure 6). Studies have proved that its effect was better than Fer-1.

According to the current findings, sorafenib caused the accumulation of lipid ROS as well as a rise of MDA and Fe⁺⁺ and a decline of GSH by the regulation of SLC7A11 and its downstream GPX4, luteolin could help release this oxidative stress by activating Nrf2 and down-regulating the expression of the associated genes HO-1 and NQO1.

Figure 6 Mechanisms of ferroptosis. Diagram of the pathway of induction and regulation of ferroptosis by sorafenib and luteolin. Fer-1, ferrostatin-1; Nrf2, nuclear factor E2-related factor 2; HO-1, heme oxygenase 1; NQO1, quinone oxidoreductase 1; GPX4, glutathione peroxidase 4; GSH, glutathione; ROS, reactive oxygen species.
Our study provides new insights into the treatment of luteolin in sorafenib-induced liver injury. However, it is crucial to verify the in-vivo efficacy of luteolin against ferroptosis-induced sorafenib. In addition, new cell lines can be added for validation in subsequent experiments.

Conclusion

In summary, this study demonstrated that luteolin reduced oxidative stress and ferroptosis, which were introduced by the negative effects of sorafenib. Therefore, luteolin can be used as an adjuvant to sorafenib to reduce liver injury in patients with hepatocellular carcinoma.

References


