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RESEARCH ARTICLE

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Curcumin attenuates isoniazid-induced hepatotoxicity by upregulating the SIRT1/PGC-1α/NRF1 pathway

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Abstract

As a serious infectious disease, tuberculosis threatens global public health. Isoniazid is the first-line drug not only in active tuberculosis but also in its prevention. Severe hepatotoxicity greatly limits its use. Curcumin, extracted from turmeric, has been found to relieve isoniazid-induced hepatotoxicity. However, the mechanism of isoniazid-induced hepatotoxicity and the protective effects of curcumin are not yet understood completely. We established both cell and animal models about isoniazid-induced hepatotoxicity and investigated the new mechanism of curcumin against isoniazid-induced liver injury. The experimental data in our study demonstrated that curcumin ameliorated isoniazid-mediated liver oxidative stress. The protective effects of curcumin were demonstrated and confirmed to be correlated with upregulating SIRT1/PGC-1α/NRF1 pathway. Western blot revealed that while inhibiting SIRT1 by the siRNA1 (a SIRT1 inhibitor), the expressions of SIRT1, PGC-1α/Ac-PGC-1α, and NRF1 decreased, and the protective effect that curcumin exerted on isoniazid-treated L-02 cells was significantly attenuated. Furthermore, curcumin improved liver functions and reduced necrosis of the isoniazid-treated BALB/c mice, accompanied by downregulating oxidative stress and inflammation in liver. Western blot revealed that curcumin treatment activates the SIRT1/PGC-1α/NRF1 pathway in the isoniazid-treated BALB/c mice. In conclusion, we found one mechanism of isoniazid-induced hepatotoxicity downregulating the SIRT1/PGC-1α/NRF1 pathway, and curcumin attenuated this hepatotoxicity by activating it. Our study provided a novel approach and mechanism for the treatment of isoniazid-induced hepatotoxicity.

KEYWORDS
curcumin, hepatotoxicity, isoniazid, protective mechanism, SIRT1/PGC-1α/NRF1 pathway

1 | INTRODUCTION

Tuberculosis is a serious infectious disease caused by *Mycobacterium* and still threatens global public health. In 2019, about 10 million people were diagnosed with tuberculosis and 1.4 million died (Harding, 2020). The emergence of isoniazid in the 1950s provided an effective way to control tuberculosis. It remains the first-line drug until now (Zumla et al., 2014) not only in active tuberculosis but also in its prevention (Nolan et al., 1999). Despite the high antibacterial activity, the severe hepatotoxicity and lethal liver injury limit its use (Metushi et al., 2016). Hence, it is urgent to reveal the hepatotoxicity mechanism and find new therapeutic targets as well as more effective approaches to prevent isoniazid-induced hepatotoxicity.

Yun Li and Wen-wen Luo have contributed equally to this work and share the first authorship.

Mitochondria, the dynamic organelle and energy center maintain energy balance, mediate apoptosis, and necrosis and regulate cell signal transduction by integrating environmental signals (Finley & Haigis, 2009; Ryan & Hoogenraad, 2007). Mitochondria in isoniazid-induced hepatotoxicity have attracted increasing attention (Hann et al., 2013; Ramachandran et al., 2018). When damage exceeds critical value that cells can tolerate, mitochondrial apoptosis and necrosis signaling pathways will be activated, and the cells die (Han et al., 2013). Although mitochondria have their own genomes, most mitochondrial proteins are encoded by the nuclear genomes (Ryan & Hoogenraad, 2007). Therefore, studying how nucleus and mitochondria communicate is crucial to understanding how mitochondria maintain their function.

Peroxisome proliferator-activated receptor-gamma coactivator 1 (PGC-1) is a family of transcriptional cofactors that regulate the expression of mitochondrial functional protein. It interacts with chromatin modifiers, transcriptional complexes, and trans-acting factors to affect the transcriptional activity of mitochondrial functional proteins (Chawla et al., 2001; Fernandez-Marcos & Auwerx, 2011; Finley & Haigis, 2009; Handschin & Spiegelman, 2006). PGC-1α has been proved to synergically activate a variety of oxidative stress-related nuclear receptors and transcription factors, including nuclear respiratory factor 1 (NRF1), nuclear respiratory factor 2 (NRF2) (St-Pierre et al., 2006; Wu et al., 1999), and forkhead box protein O1 (FoxO1) (Puigserver et al., 2003). NRF1 and NRF2 bind to antioxidant response elements (ARE) in the promoter region, which will promote the expression of downstream-related genes to combat oxidative stress injury.

Silent information regulator 1 (SIRT1), an NAD^+-dependent class III histone deacetylase, regulates a huge number of biological processes including metabolism, aging, oxidative stress, apoptosis, and inflammation (Yan et al., 2019). It cooperates with histone acetylase (GCN5) to regulate the transcriptional activity of PGC-1α (Canto et al., 2009; Canto & Auwerx, 2009; Fernandez-Marcos & Auwerx, 2011). Low energy state activates adenosine monophosphate-activated protein kinase (AMPK), which increases the content of NAD^+. Increasing NAD^+ then enhances the activity of SIRT1, which catalyzes the deacetylation of Ac-PGC-1α. On the contrary, GCN5 catalyzes the acetylation of PGC-1α at a high energy state and inhibits the transcriptional activity of PGC-1α. Therefore, SIRT1/GCN5 can change transcriptional activity of PGC-1α under different energy states. In other words, they transform the energy change into the change of gene expression. Therefore, PGC-1α is called a metabolic sensor (Spiegelman & Heinrich, 2004).

Curcumin, derived from a ginger family called *Curcuma Longa L.*, has been found to have antioxidative, anti-inflammatory, anti-cancer, anti-bacterial, anti-atherosclerosis, anti-liver fibrosis, anti-coagulation, and other pharmacological activity (Zhu et al., 2017). Clinical trials showed that concurrent administration of anti-tuberculosis drugs with curcumin could significantly reduce the incidence and severity of drug-induced liver injury (DILI) (Adhvaryu et al., 2008). Similarly, it has been confirmed by so many in vitro/in vivo experiments that curcumin promoted the expression of antioxidative genes by activating the Nrf2/ARE pathway (Farzaei et al., 2018; Gao et al., 2013; Ikram et al., 2019). However, there is a lack of evidence for upstream mechanisms, such as the SIRT1/PGC-1α pathway, and NRF1 and NRF2, which also initiate transcription of ARE. Cell experiments showed that curcumin promoted the production of PGC-1α in tumor cells by activating the AMPK pathway. Animal experiments showed that curcumin reduced mitochondrial damage in skeletal muscle of COPD rats, and this protective effect is also achieved through activation of the PGC-1α-related pathway (Zhang, Ikejima, et al., 2017).

In this study, we investigated the mechanism of isoniazid-induced hepatotoxicity and the protective effects of curcumin in vitro and vivo. Study showed that silibinin could protect the liver by upregulating the level of NAD^+ and activate AMPK/SIRT1 pathway (Salomone et al., 2017). Therefore, this study intends to use silibinin as a positive control and compare curcumin effects with it.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Curcumin (Cat No. 71012660, purity > 99%) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). It was dissolved in 0.2% dimethyl sulfoxide (DMSO) in vitro and in olive oil containing 3% DMSO for in vivo. Isoniazid (Cat No. I3377, purity ≥ 99%) was bought from Sigma Aldrich Co. (St. Louis, USA). It was dissolved in phosphate buffer saline (PBS) in vitro and in saline in vivo. Silibinin (Cat No. S0417, purity ≥ 98%) was bought from Sigma Aldrich Co. (St. Louis, USA). It was dissolved in 0.2% dimethyl sulfoxide (DMSO) for in vitro experiments. Antibody against SIRT1 (Cat No. 60303-1-Ig) and β-actin (Cat No. 66009-1-Ig) were purchased from Proteintech (Chicago, USA); antibody against NRF1 (Cat No. A3252) were purchased from Abclonal Technology (Wuhan, China); antibody against PGC-1α (Cat No. sc-517380) were purchased from Santa Cruz Biotechnology (Dallas, USA). RPMI medium 1640 basic, fetal bovine serum, penicillin streptomycin, and 0.25% Trypsin–EDTA were purchased from Gibco (New York, USA). Glycine, Tris, and SDS were purchased from Meilunbio (Dalian, China). Acr, Bis, AP, TEMED, and Tris–HCl were purchased from Servicebio (Wuhan, China). Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), reduced glutathione (GSH), malondialdehyde (MDA), and reactive oxygen species (ROS) assay kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). RIPA lysis buffer, BCA protein assay kit, and phenyl methane sulfonyl fluoride (PMSF) were purchased from Boster Biological Technology Co., Ltd. (Wuhan, China).

2.2 | Cell culture

L-02 cells (Shanghai Zhong Qiao Xin Zhou Biotechnology Co., Ltd., China) were grown in RPMI 1640 with 10% fetal bovine serum, 100 units/ml penicillin G, and 100 μg/ml streptomycin in an incubator at 37°C and 5%CO_2. All cells were passaged using trypsin.

2.3 | CCK-8 assay for cell viability

L-02 cells were plated in 96-well plates at a density of 5×10^3cells per well in a 0.1 ml complete medium. After 24 h incubation, the medium was replaced with isoniazid and (or) curcumin or silibinin at different concentrations for 12, 24, and 48 h. After that, each well was incubated with 10% CCK-8 solution (Beyotime Co. Shanghai, China) at 37°C for 2 h. The incubated plate was then placed into a microplate reader to determine the optical density (OD) value at the wavelength of 450 nm. The OD of the formazan generated by dehydrogenases is directly proportional to the number of viable cells.

2.4 | SiRNA transfection

L-02 cells were plated in 6-well plates. SiRNA (100 nM) and lipofectamine 3000 (Invitrogen) were diluted in reduced serum medium (Gibco) and transfected according to the manufacturer's protocol. After 6 h of transfection, the medium was replaced with RPMI 1640 containing 10% FBS and continually incubated for 12 h. Then cells were treated with curcumin or silibinin for an additional 12 h. Last, the cells were treated with combined curcumin or silibinin and isoniazid for an additional 24 h. Protein expression was measured by western blot and immunoprecipitation. Target sequences of the SIRT1 siRNA used in this study are shown in Table 1 (RiboBio Co., Ltd., Guangzhou, China).

2.5 | Determination of intracellular ROS

The intracellular ROS was measured using a fluorescent probe DCFH-DA. After drug intervention, cells were washed with PBS and incubated with 10 μmol/L DCFH-DA at 37°C for 1 h in dark. Then the cells were washed three times with PBS to remove the free probe. The fluorescence intensity of intracellular ROS was observed by fluorescence microscopy and photographed (IX-73; Olympus, Tokyo, Japan). Finally, fluorescence intensity was compared by observation.

2.6 | Animals

Male BALB/c mice (20 ± 2 g, 6–8 weeks) were purchased from Hunan SJA Laboratory Animal Co. Ltd (Hunan, China). They were kept in cages under standard colony conditions with constant temperature (22°C), humidity (50 ± 10%), and a 12 h light/dark circle. All animals were fed with standard feed, and mineral water was made available casually through nonpolysulfone bottles. Seven days after adapting, the mice were randomly divided into four groups (eight in each group): isoniazid/curcumin group, isoniazid group, curcumin group, and control group. Curcumin was dissolved in special solvent (olive oil containing 3% DMSO), whereas isoniazid was dissolved in saline. Both curcumin and isoniazid were administered intragastrically. Mice in isoniazid/curcumin group were administered with curcumin followed by isoniazid 30 min later. Mice in isoniazid group were administered with an equal volume of solvent followed by isoniazid. Mice in curcumin group were administered with curcumin followed by saline. Mice in control group were administered with solvent followed by saline. Isoniazid or curcumin was administered intragastrically at doses of 100 mg/kg once daily. All treatments were performed for 28 consecutive days. Following 24 h fasting after the final treatment, animals were anesthetized and sacrificed. Blood samples and liver tissues were collected. All the procedures were based on the guidelines, and the animal experiment protocol was approved by the Ethics Committee of Experimental Animal Welfare of Central South University (ethics code: 2020sydw0808).

TABLE 1 Target sequences for SIRT1 siRNA used in the study

Name of sequence	Catalog number	Target sequence
SIRT1 siRNA1	stB0002824A	GCCTGATGTTCCAGAGAGA
SIRT1 siRNA2	stB0002824B	GACATGAACTATCCATCAA
SIRT1 siRNA3	stB0002824C	GGATGAAAGTGAAATTGAA

2.7 | Determination of serum biochemical indexes and hepatic antioxidase activities

The liver index was calculated according to the formula: liver index (%) = mouse liver weight (g)/mouse weight (g) × 100%. The serum biochemical indexes, such as ALT, AST, ALP, LDH, TBA, and TBIL, were measured by absorbance photometry using the automatic analyzer. RIPA lysis buffer containing 1% PMSF was used to extract liver soluble substance. The whole lysis buffer was centrifuged with 12,000 rpm at 4°C for 15 min. The supernatant was collected, and protein concentration was measured by bicinchoninic acid (BCA) method according to the manufacturer's protocol. And then, the concentration of MDA and GSH and activity of SOD in supernatant were detected using commercial kits according to the manufacturer's instructions. All samples were operated parallel for three times. The results were obtained by microplate reader and were displayed as U per milliliter for SOD and μmol per gram for MDA and GSH. U is the international unit of enzyme, which represents the amount of enzyme required to convert 1 mmol substrate in 1 min.

2.8 | Histopathology

A small piece of left lobe of liver was cut and fixed with 4% paraformaldehyde, then paraffin embedded. Paraffin blocks were cut into thin sections and stained with hematoxylin and eosin (H&E), then analyzed under the optical microscope.

2.9 | Western blot

RIPA lysis buffer containing 1% PMSF was used to extract total protein of liver or cells. The whole lysis buffer was centrifuged with 12,000 rpm at 4°C for 15 min. The supernatant was collected, and protein concentration was measured by BCA method. Samples (equal to 20 mg total protein per lane) were separated by electrophoresis on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel, then transferred to PVDF membranes followed by blocking with 5% skimmed milk for 1 h at room temperature. PVDF membranes were cut according to the ladder, and incubated with the targeted primary antibodies (SIRT1, 1:4000; PGC-1α, 1:400; NRF1, 1:500; β-actin, 1:5000) at 4°C overnight. After 16–18 h, the PVDF membranes were washed three times with tris-buffered saline tween (TBST). After washing, the membrane was incubated with suitable HRP-conjugated secondary antibodies (Cat No. SA00001-1, goat anti-mouse IgG; Cat No. SA00001-2, goat anti-rabbit immunoglobulin IgG, Proteintech, USA) at room temperature for 1 h. Finally, after washing three times with TBST, the target proteins were detected using hypersensitive ECL chromogenic solution (Servicebio, Wuhan, China). The results were analyzed using ImageJ software.

2.10 | Immunoprecipitation

RIPA lysis buffer containing 1% PMSF was used to extract total protein of liver or cells. The whole lysis buffer was centrifuged with 12,000 rpm at 4°C for 15 min. Each 500 μl supernatant was added with 0.25 μg rabbit control IgG (Abclonal Technology, Wuhan, China) and 20 μl Protein A/G PLUS-Agarose (Santa Cruz Biotechnology, Dallas, USA). Gently stirred and incubated at 4°C for 30 min, the mixed system was centrifuged with 3000 rpm at 4°C for 30 s. The supernatant was collected and adjusted to the same concentration with RIPA lysis buffer containing 1% PMSF. Later, the supernatant was added with 2 μl Pan Acetyl-Lysine Rabbit pAb (Cat No. A2391, Abclonal Technology, Wuhan, China) followed by incubating at a shaker at 4°C for 2 h, and 20 μl Protein A/G PLUS-Agarose followed by incubating at a shaker at 4°C overnight. The supernatant was centrifuged with 3000 rpm at 4°C for 30 s. After that, the supernatant was discarded, and the sediment (mainly agarose-antibody–antigen complexes) was resuspended with 1 ml RIPA lysis buffer. The resuspension step was repeated three times, and the agarose was resuspended with 20 μl loading buffer followed by boiling for 10 min. At last, the loading buffer was centrifuged at 3000 rpm and 4°C for 30 s. The supernatant was collected and stored at −20°C. The next step was western blot analysis, which has been described in Section 2.9.

2.11 | Statistics

All data were expressed as mean ± standard deviation. The data were analyzed by SPSS 24.0 through one-way ANOVA followed by Dunnett's *t* test or LSD *t* test. $P < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Curcumin and silibinin protect L-02 cells from isoniazid-induced cytotoxicity

To determine whether curcumin protects L-02 cells from isoniazid-induced cytotoxicity, we used CCK-8 to test cell viability. First, L-02 cells were exposed to isoniazid at a series of concentration gradients (5–120 mmol/L) for three intervention time (12, 24, and 48 h). The results revealed that cell viability decreased in a dose- and time-dependent manner, so we chose 20–40 mmol/L together with 24 h for further study (Figure 1A). Next, the effect of curcumin or silibinin on cell viability was tested (Figure 1B,C). L-02 cells were exposed to different concentration of curcumin (1–80 μmol/L) or silibinin (2.5–200 μmol/L) for three intervention time (12, 24, and 48 h). When the dose was more than 10 μmol/L, curcumin and silibinin reduced the cell viability in a dose-dependent manner. Therefore, we chose 0.5–10 μmol/L curcumin and silibinin for further study. Last, we pretreated L-02 cells with the indicated five doses of curcumin (0.5, 1, 2, 5, and 10 μmol/L) or silibinin (0.5, 1, 2, 5, and 10 μmol/L) for 12 h, then treated curcumin or silibinin combined with isoniazid (20–40 mmol/L) for 24 h (Figure 1D,E). The control group was treated with complete medium for 12 h, and 20–40 mmol/L isoniazid for the last 24 h. Surprisingly, besides 10 μmol/L curcumin, other doses of curcumin and silibinin slightly affected cell viability. As a result, we selected 0.5–5 μmol/L curcumin and 1–10 μmol/L silibinin combined with 40 mmol/L isoniazid for subsequent experiments.

3.2 | Curcumin and silibinin decrease ALT, AST, LDH, and ROS levels in isoniazid-treated L-02 cells

The effects of curcumin or silibinin combined with isoniazid on ALT, AST, LDH, and ROS were shown in Figure 2. Levels of ALT, AST, and LDH decreased gradually as doses of curcumin or silibinin increased, indicating a growing protective effect (Figure 2A). After combining 5 μmol/L curcumin and 40 mmol/L isoniazid, levels of ALT, AST, and LDH decreased to 4.95, 28.18, and 50.90 U/L, which were 12.94%, 45.54%, and 74.44% of those treated with isoniazid only. With the combination of 10 μmol/L silibinin and 40 mmol/L isoniazid, levels of these three indexes decreased to 3.70, 26.85, and 49.24 U/L, which were 9.67%, 43.39%, and 72.01% of those treated only with isoniazid. The level of ROS also decreased gradually with the increasing doses of curcumin or silibinin. As shown in Figure 2B, the intensity of intracellular green fluorescence gradually decreased from left (low doses) to right (high doses), indicating a gradual decrease of ROS levels. Finally, we selected 2–5 μmol/L curcumin and 5–10 μmol/L silibinin for following study.

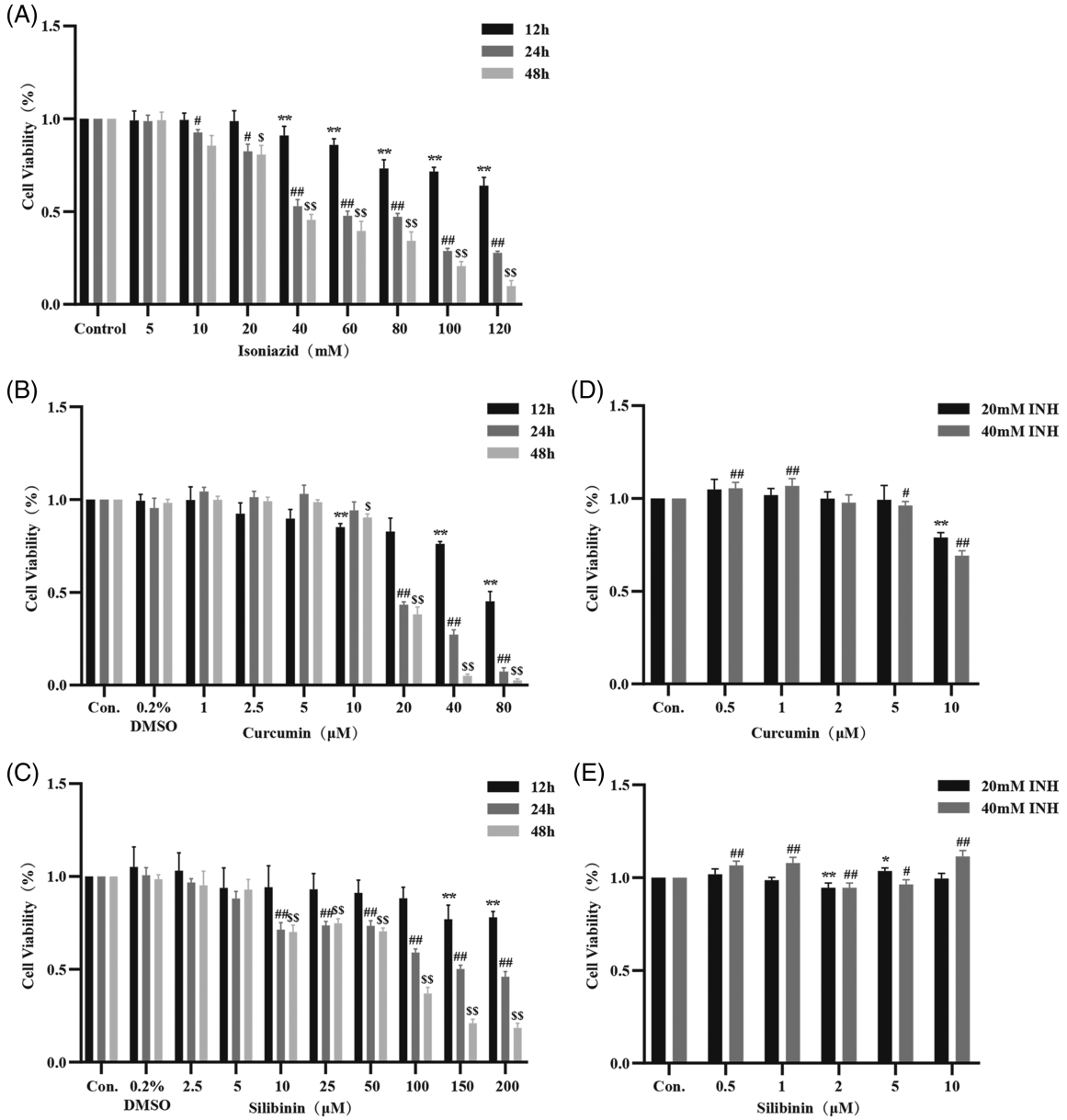

FIGURE 1 Curcumin and silibinin improved isoniazid-induced cell viability loss in L-02 cells. (A) Cell viability after exposed to isoniazid (5, 10, 20, 40, 60, 80, 100, and 120 mmol/L) for 24 or 48 h. (B, C) Cell viability after exposed to curcumin (1–80 μmol/L) or silibinin (2.5–200 μmol/L) for 12, 24, or 48 h. $^{**}P < 0.01$ vs. Con. Group (12 h). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Con. Group (24 h). $^{\$}P < 0.05$, $^{\$\$}P < 0.01$ vs. Con. Group (48 h). (D, E) Cell viability after pretreated with curcumin (0.5, 1, 2, 5, and 10 μmol/L) or silibinin (0.5, 1, 2, 5, and 10 μmol/L) for 12 h, then combined with isoniazid (20/40 mmol/L) for 24 h. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. Con. Group (20 mmol/L). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. Con. Group (40 mmol/L). The Con. Group was operated parallel with phosphate buffer saline. Con., control; INH, isoniazid. Data were represented as mean ± SD ($n = 4$)

3.3 | Curcumin and silibinin upregulate SIRT1/PGC-1α/NRF1 pathway in isoniazid treated L-02 cells

To determine how curcumin affect the SIRT1/PGC-1α/NRF1 pathway in isoniazid treated L-02 cells, we used western blot to analyze the expression of these proteins. It showed that the contents of SIRT1, PGC-1α, and NRF1 were distinctly downregulated in response to isoniazid treatment, whereas these effects were partially reversed by curcumin and silibinin (Figure 3). Compared with control group (−/−/−), SIRT1, PGC-1α, and NRF1 in isoniazid group (−/−/40) were significantly decreased to 61%, 34%, and 45% of those in control group, respectively. Compared with isoniazid group, the contents of SIRT1, PGC-1α, and NRF1 in combination group were significantly increased, and the extent of increase had a positive correlation with the doses of curcumin and silibinin. The contents of SIRT1, PGC-1α, and NRF1 in 5 μmol/L curcumin group (5/−/40) were 1.52, 1.98, and 1.78 times higher than those in isoniazid group, respectively. In 10 μmol/L silibinin group (−/10/40), the contents of these three proteins were increased to 1.77, 1.87, and 2.32 times, respectively. Results in this part proved that the

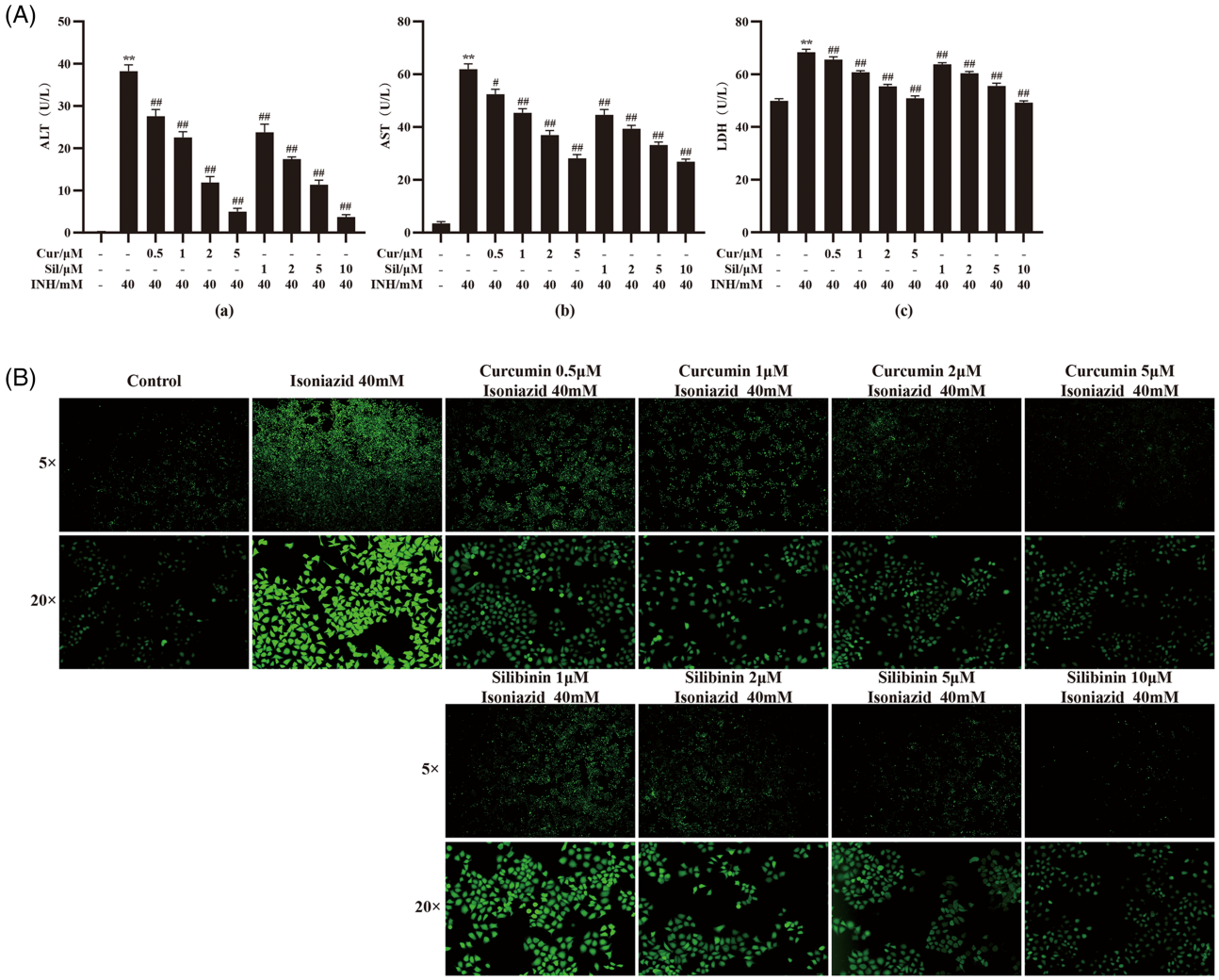

FIGURE 2 Curcumin and silibinin decreased levels of ALT, AST, LDH, and ROS in isoniazid-treated L-02 cells. (A) Levels of ALT, AST, and LDH in cell supernatant after exposed to curcumin (0.5–5 μmol/L) or silibinin (1–10 μmol/L) for 12 h and then combined with isoniazid (40 mmol/L) for 24 h. Data were represented as mean ± SD ($n = 4$); $^{**}P < 0.01$ vs. (−/−/−) group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. (−/−/40 mmol/L INH) group. (B) The level of ROS in L-02 cells after pretreating with curcumin (0.5–5 μmol/L) or silibinin (1–10 μmol/L) for 12 h, then combined with isoniazid (40 mmol/L) for 24 h. The brighter the green fluorescence, the higher the ROS content in the cell. INH, isoniazid; Cur, curcumin; Sil, silibinin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; ROS, reactive oxygen species

protective effects of curcumin might be associated with SIRT1/PGC-1α/NRF1 pathway.

3.4 | Inhibition of SIRT1/PGC-1α/NRF1 pathway weakens the protective effects of curcumin or silibinin in isoniazid treated L-02 cells

To make clear whether the protective effects of curcumin or silibinin were associated with SIRT1/PGC-1α/NRF1 pathway or not, we transfected L-02 cells with SIRT1 siRNA, which could silence the expression of SIRT1. We used three different siRNA sequences and compared these sequences with the control group and negative control group (only transfected with blank carrier). The transfection efficiencies of three sequences were 85.3%, 73.6%, and 81.8%, respectively (Figure 4). SIRT1 siRNA1 with the highest efficiency was selected for subsequent experiments. When SIRT1 silenced, change of SIRT1/PGC-1α/NRF1 pathway is shown in Figure 5A. The contents of SIRT1, PGC-1α/Ac-PGC-1α ratio, and NRF1 all decreased after SIRT1 silenced. Before SIRT1 silenced, as is mentioned in Section 3.3, curcumin and silibinin upregulated SIRT1/PGC-1α/NRF1 pathway. In reverse, when SIRT1 silenced, this upregulation effect was mostly inhibited. As a result, curcumin and silibinin could not decrease the levels of ALT, AST, LDH, and ROS in isoniazid treated L-02 cells (Figure 5B,C). Together, these results indicate that the SIRT1/PGC-1α/NRF1 pathway is highly associated with the protective effects of curcumin and silibinin in isoniazid-related hepatotoxicity.

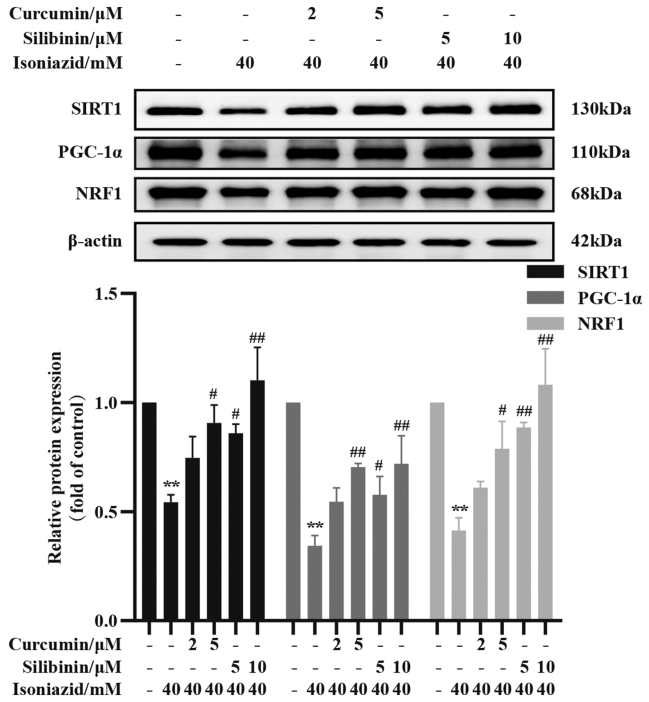

FIGURE 3 Curcumin and silibinin upregulated SIRT1, PGC-1α, and NRF1 proteins expression in isoniazid-treated L-02 cells. L-02 cells were pretreated with curcumin (2–5 μmol/L) or silibinin (5–10 μmol/L) for 12 h and then treated with isoniazid (40 mmol/L) for 24 h to detect the protein levels of SIRT1, PGC-1α, and NRF1 in the cells. Data were represented as mean ± SD ($n = 3$); $^{**}P < 0.01$ vs. control group (−/−/−); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. isoniazid group (−/−/40)

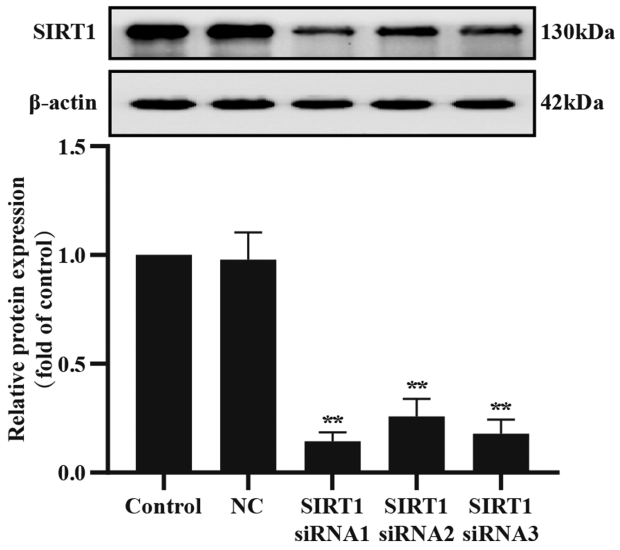

FIGURE 4 SIRT1 siRNA transfection efficiency. L-02 cells were transfected with negative control or SIRT1 siRNA1/2/3 for 6 h, respectively, to detect the expression of SIRT1 protein in the cells. Compared with the NC group, $^{**}P < 0.01$. ($n = 3$). NC, negative control

3.5 | Curcumin alleviates isoniazid-induced hepatotoxicity in BALB/c mice

To make clear the protective effects of curcumin against isoniazid-induced hepatotoxicity in vivo, we designed the animal experiment. The results of ALT, AST, ALP, LDH, TBA, and TBIL (all of these are vital indicators of hepatotoxicity in the clinic) were shown in Figure 6A. The levels of ALT, AST, ALP, LDH, and TBIL were increased in isoniazid group (Sol + INH), which were decreased by curcumin in reverse. Besides, the levels of TBA had no significant difference among all groups. Results of liver index assessment showed that administration of isoniazid resulted in a significantly increased liver index, whereas curcumin improved it (Figure 6B). H&E staining assays were used to evaluate the liver histopathology (Figure 6C). The isoniazid group showed obvious necrosis and inflammatory cell infiltration, whereas the combined group (Cur + INH) showed the liver protection from isoniazid-induced damage.

3.6 | Curcumin reduces isoniazid-induced oxidative stress in BALB/c mice

To investigate the protective effects of curcumin against isoniazid-induced oxidative stress, we evaluated the level of GSH, SOD, and MDA in the liver tissue. As shown in Figure 7, the GSH content and SOD activity were markedly decreased after isoniazid treatment. However, curcumin treatment increased them. The (A) GSH and (B) SOD in isoniazid group (Sol + INH) decreased to 56% and 87% of those in control group (Sol + Sal), respectively ($P < 0.01$). But in the combined group (Cur + INH), these two indexes increased by 1.41 times and 1.11 times compared with the isoniazid group, respectively ($P < 0.05$). MDA showed no significant difference among all groups.

3.7 | Curcumin upregulates the SIRT1/PGC-1α/NRF1 pathway against isoniazid-induced hepatotoxicity in BALB/c mice

To identify the role of the SIRT1/PGC-1α/NRF1 pathway in isoniazid-induced hepatotoxicity in BALB/c mice, protein contents of SIRT1, PGC-1α, and NRF1 were measured by western blot, whereas Ac-PGC-1α was measured by immunoprecipitation. As shown in Figure 8, isoniazid significantly decreased SIRT1, PGC-1α/Ac-PGC-1α, and NRF1, whereas curcumin elevated them. Compared with the control group (Sol + Sal), (A) SIRT1 and (B) NRF1 in the isoniazid group (Sol + INH) were significantly decreased to 49% and 50% of that in the control group ($P < 0.01$), and decreased PGC-1α and increased Ac-PGC-1α causing the ratio of PGC-1α/Ac-PGC-1α to decrease significantly to 15% of that in the control group ($P < 0.01$). The overall downregulation indicated isoniazid had an inhibitory effect on the SIRT1/PGC-1α/NRF1 pathway. On the contrary, curcumin significantly increased SIRT1 and NRF1, which were 1.81 and 2.15 times higher than that in the isoniazid group ($P < 0.05$). Meanwhile, the ratio

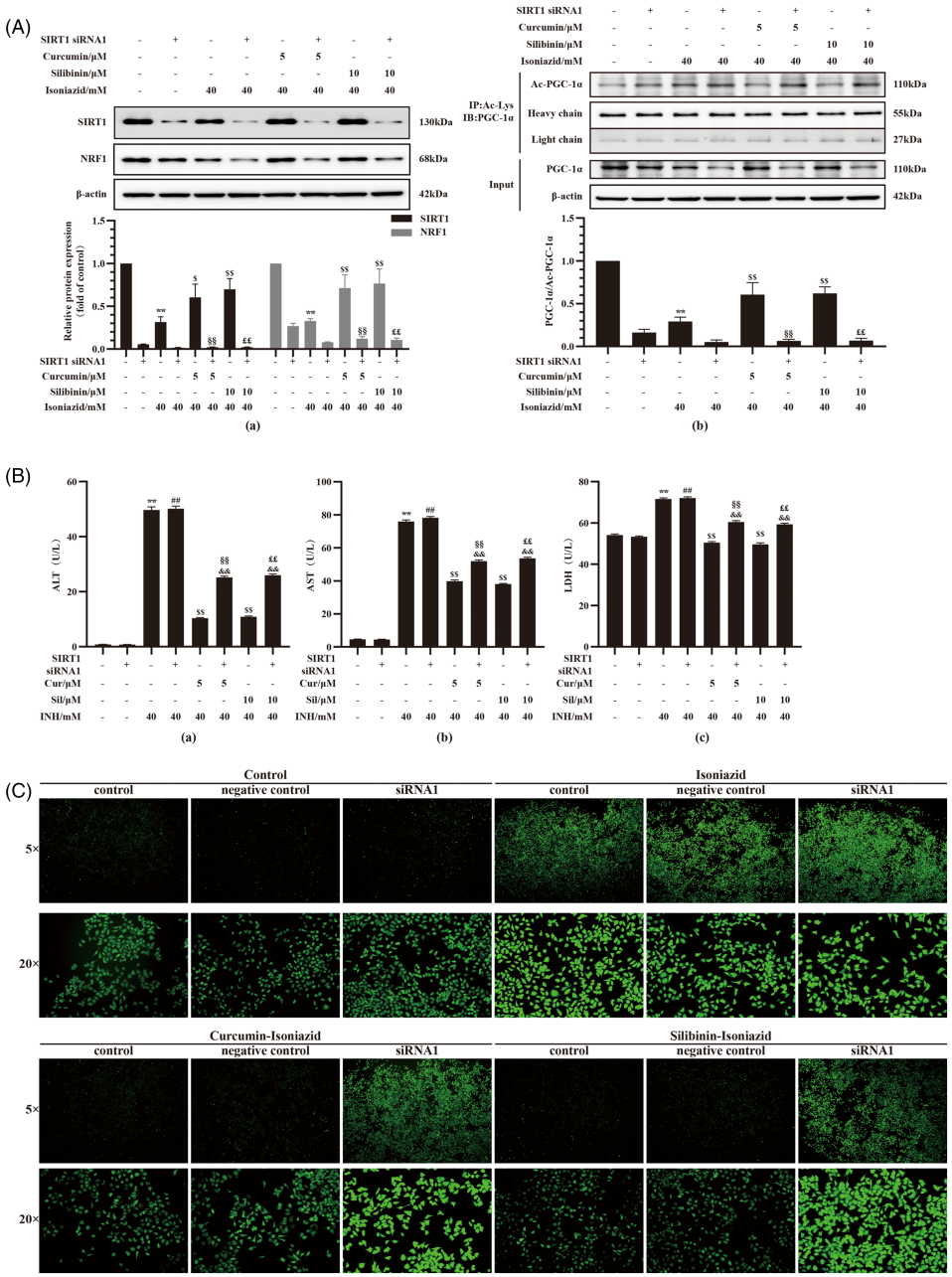

FIGURE 5 Effects of curcumin and silibinin on isoniazid treated L-02 cells after SIRT1 silencing. (A) The protein expression of SIRT1, PGC-1α, Ac-PGC-1α, and NRF1. $^{**}P < 0.01$ vs. (−/−/−/−) group; $^{\$\$}P < 0.01$ vs. (−/−/−/40) group; $^{\S\S}P < 0.01$ vs. (−/5/−/40) group; $^{\pounds\pounds}P < 0.01$ vs. (−/−/10/40) group. (B) Levels of ALT, AST, and LDH in cell supernatant. $^{**}P < 0.01$ vs. (−/−/−/−) group, $^{\#\#}P < 0.01$ vs. (+/−/−/−) group, $^{\$\$}P < 0.01$ vs. (−/−/−/40) group, $^{\&\&}P < 0.01$ vs. (+/−/−/40) group, $^{\S\S}P < 0.01$ vs. (−/5/−/40) group, $^{\pounds\pounds}P < 0.01$ vs. (−/−/10/40) group. Data were represented as mean ± SD ($n = 4$). (C) ROS content in the cell. INH, isoniazid; Cur, curcumin; Sil, silibinin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; ROS, reactive oxygen species

of PGC-1α/Ac-PGC-1α significantly increased by 4.93 times that of the isoniazid group ($P < 0.01$).

4 | DISCUSSION

The use of isoniazid to treat or prevent tuberculosis is often accompanied by severe liver injury. Being forced to stop anti-tuberculosis may cause disease reappear. Thus, we intend to explore effective drugs or compounds against isoniazid-induced liver injury. Herein, we showed that curcumin could alleviate isoniazid-induced hepatotoxicity both in vitro (L-02 cells) and in vivo (in BALB/c mice), which is associated with the upregulation of the SIRT1/PGC-1α/NRF1 pathway. Our study provided a novel approach and mechanism for the treatment of isoniazid-induced hepatotoxicity.

In this study, curcumin and silibinin show a protective effect on L-02 cells, and can reduce ALT, AST, LDH and ROS, which is consistent with the results of this project (Gao et al., 2013; He et al., 2017). In vivo, we measured serum biochemical indicators and evaluated liver pathology. We observed that the activity of ALT and AST significantly increased under isoniazid treatment, which was accompanied by typical morphological changes, including obvious vacuolization and infiltration of inflammatory cells. Contrary to isoniazid, when used in combination, curcumin substantially reduced serum indicators and improved pathological lesion. There was evidence that oxidative stress was responsible for the hepatotoxicity of isoniazid (Verma

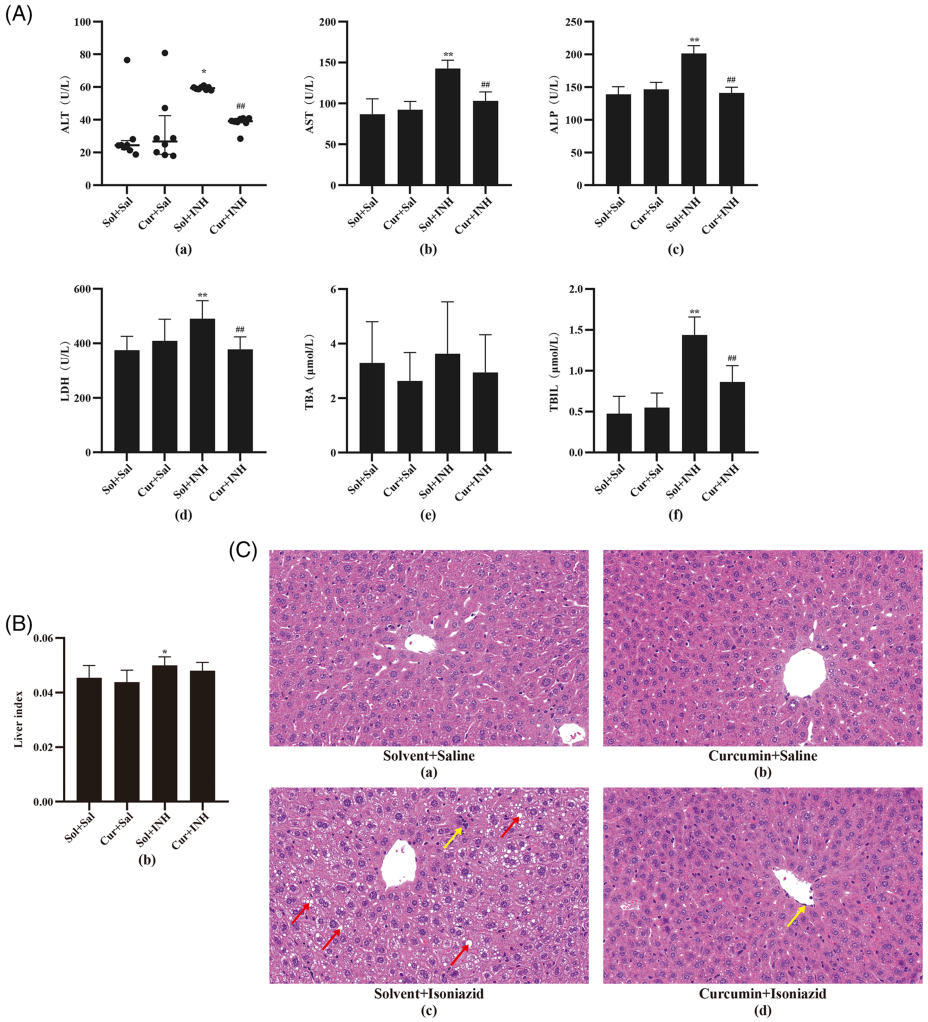

FIGURE 6 Effect of curcumin on liver function tests, liver index, and liver histopathology. Mice were administrated with 100 mg/kg isoniazid and 100 mg/kg curcumin for 28 days. After mice were sacrificed, the liver was collected. (A) Liver function was tested. $^{\#\#}P > 0.05$, $^{**}P < 0.001$. (B) The liver index of each group was measured. $^{*}P < 0.05$. (C) Liver histopathological examination was performed by using H&E staining (original magnification ×400). ($n = 8$) ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; TBA, total bile acid; TBIL, total bilirubin; INH, isoniazid; Cur, curcumin; H&E, hematoxylin and eosin

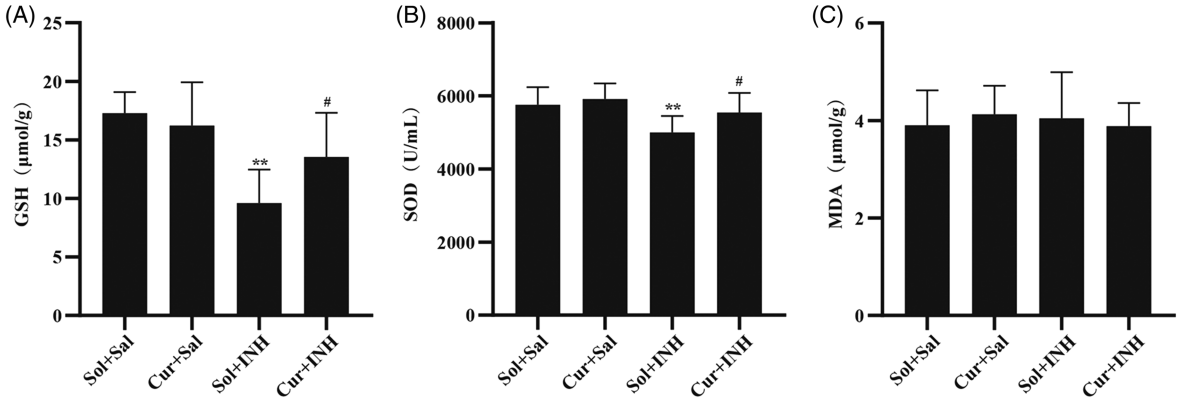

FIGURE 7 Effect of curcumin on oxidative stress in the isoniazid-induced hepatotoxicity BALB/c mice model. After the mice were sacrificed, the liver was collected and oxidative stress-related biomarkers including (A) GSH, (B) SOD, and (C) MDA were determined. Data were represented as mean ± SD ($n = 8$), $^{\#}P > 0.05$, $^{**}P < 0.001$. Sol, the solvent of curcumin; Sal, saline; INH, isoniazid; Cur, curcumin; GSH, glutathione; SOD, superoxide dismutase; MDA, malondialdehyde

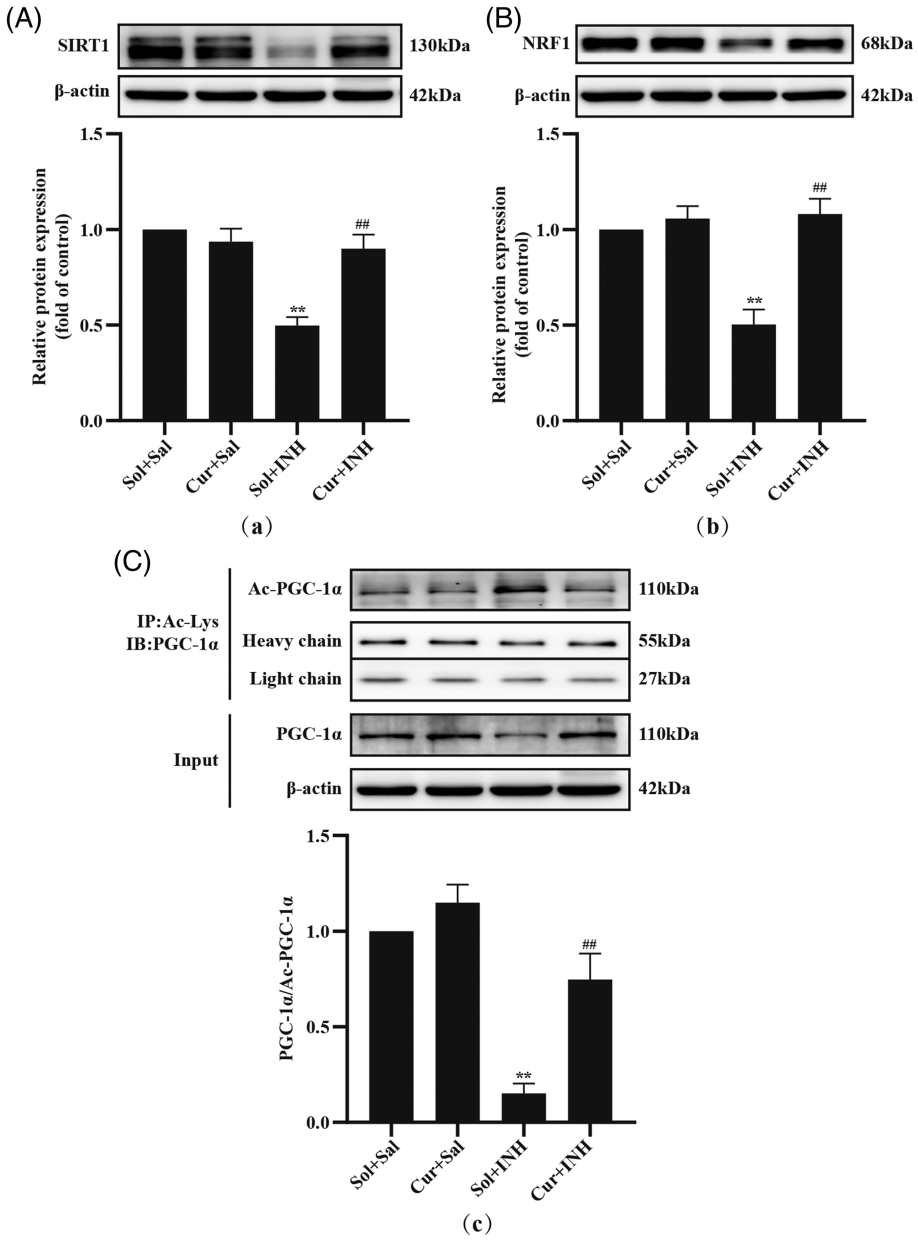

FIGURE 8 Effects of curcumin on the expressions of SIRT1, PGC-1α, Ac-PGC-1α, and NRF1 in the BALB/c mouse model. After the mice were sacrificed, the liver was collected. Western blot and immunoprecipitation were applied to determine the protein expressions of SIRT1, PGC-1α, Ac-PGC-1α, and NRF1 in the liver. Data were represented as mean ± SD ($n = 8$), $^{\#\#}P > 0.05$, $^{**}P < 0.01$. Sol, the solvent of curcumin; Sal, saline; INH, isoniazid; Cur, curcumin

et al., 2018). In this study, the antioxidant enzymes GSH and SOD significantly decreased after isoniazid exposure. Similarly, curcumin reversed the situation, which concluded that curcumin could effectively ameliorate isoniazid-induced hepatic oxidative stress. The result of MDA showed no significant difference. We speculated that one of the reasons may be that the mice adapted to the injury after long-term administration, and the other may be that the isoniazid dose was insufficient, leading an unobvious damage. Lian's group applied 75/150/300 (mg/kg/day) isoniazid for consecutive 15 days, but only 150/300 (mg/kg/day) groups showed increased MDA (Lian et al., 2017).

A series of studies have shown that SIRT1 has critical roles in ameliorating hepatotoxicity by inhibiting oxidative stress, inflammation, and apoptosis by interacting with other signaling pathways (Nagappan et al., 2019; Song et al., 2019; Tian et al., 2016; Yuan et al., 2020). This study proved that the antioxidant property of curcumin in isoniazid-induced hepatotoxicity was associated with upregulating SIRT1/PGC-1α/NRF1 pathway. Isoniazid downregulated the SIRT1/PGC-1α/NRF1 pathway, while curcumin upregulated it. After SIRT1 silenced, the effect of curcumin was partially inhibited. This conclusion is consistent with many other studies, which had confirmed that isoniazid inhibited SIRT1 expression in HepG2 and HL7702 cells and downregulated SIRT1/PGC-α/NRF1 pathway (Zhang, Li, et al., 2019; Zhang, Tang, et al., 2017; Zhang, Zhang, et al., 2019). Similarly, in 2018, Portale-Perez DP's team found that, with SIRT1 inhibited, the activity of NAT2 increased; with SIRT1 stimulated, the result was quite opposite (Salazar-Gonzalez et al., 2018; Turijan-Espinoza et al., 2018). Because the increased activity of NAT2 is prone to isoniazid-induced hepatotoxicity, study mentioned above confirmed our study from the side and put forward another possibility

for isoniazid-induced hepatotoxicity. In other disease such as diabetes mellitus with myocardial injury or osteoarthritis, curcumin had been proven to be protective through activating SIRT1 (Feng et al., 2019; Li et al., 2019). In this study, we confirmed that curcumin played a protective role against isoniazid-induced hepatotoxicity through activating SIRT1.

Curcumin had been proved to be a regulator of SIRT1, promoting the deacetylation of multiple downstream acetylated proteins, including Ac-FoxO1, Ac-PGC-1α, and Ac-p53 (Feng et al., 2019; Miao et al., 2016; Ren et al., 2020). PGC-1α is converted from Ac-PGC-1α by deacetylation and regulates NRF1 transcription (St-Pierre et al., 2006; Wu et al., 1999). NRF1 was reported to shift into the nucleus and start the transcription of various ARE-related genes in response to oxidative stress injury (Bea et al., 2003; Campbell et al., 2013; Hou et al., 2018; Loboda et al., 2016; Weerachayaphorn et al., 2009). In this study, we confirmed that curcumin plays a protective role by upregulating SIRT1/PGC-1α/NRF1 pathway. However, the intermediate mechanism by which curcumin regulates SIRT1 still needs to be further studied.

As an important kinase, AMPK regulates metabolic homeostasis in vivo. It has been proved that ROS can directly and reversibly modify the active cysteine sulfhydryl (-SH) on the α and β subunits of AMPK, inhibiting its activity (Filomeni et al., 2015; Zhao et al., 2017). And it also has been shown that AMPK indirectly regulates the SIRT1 expression by regulating the NAD^+/NADH ratio (Canto et al., 2009). In this study, we detected the intracellular ROS production and found that curcumin could reduce intracellular ROS, with which we could speculate that curcumin may promote the recovery of AMPK, and thus promote the expression of downstream SIRT1 (Lin et al., 2015; Patel et al., 2020; Ray Hamidie et al., 2015). In summary, curcumin potentially regulates the SIRT1/PGC-1α/NRF1 pathway by regulating ROS/AMPK/NAD^+ pathway which requires further study.

5 | CONCLUSION

Curcumin has a protective effect on isoniazid-induced hepatotoxicity by suppressing oxidative stress and inflammation. We found one mechanism of isoniazid-induced hepatotoxicity downregulating the SIRT1/PGC-1α/NRF1 pathway, and curcumin attenuated this hepatotoxicity by activating it. Our study provided a novel approach and mechanism for the treatment of isoniazid-induced hepatotoxicity.

CONFLICT OF INTEREST

The authors did not report any conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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