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Chelerythrine-mediated targeting of NF-κB and Nrf2 pathways alleviates liver injury in a carbon tetrachloride-induced liver fibrosis mouse model

Yisong Ding[1]*, Xiaoming Li[2]*, Ruixing Qi[1], Yingshi Su[3] and Xiaoli Wang[1]

[1]School of Pharmacy, [2]The Institute of Medicine and [3]Scientific Research Department, Qiqihar Medical University, Qiqihar, China

*Xiaoming Li and Yisong Ding contributed equally to this work

Summary. Background and Aims. This study aimed to investigate the mechanism and efficacy of chelerythrine (CHE) in treating carbon tetrachloride (CCl_4)-induced liver fibrosis, with a particular focus on the nuclear factor-erythroid-related factor-2 (Nrf2) and nuclear factor-kappa-B (NF-κB) signaling pathways.

Methods. Mice were induced with CCl_4 for eight weeks and categorized into the control group, CCl_4 model group, and CHE low (7 mg/kg/d, ig,), medium (14 mg/kg/d, ig), and high-dose (28 mg/kg/d, ig) groups with 10 animals in each group. Following CHE treatment, liver sample morphology was assessed using multiple immunohistochemistry, and serum biochemical indicators were measured. ELISA was used to determine IL-10, IL-1β, and TNF-α contents. Western blotting and RT-PCR were employed to analyze protein and mRNA levels of α-SMA, Col-I, fibronectin, Nrf2, HO-1, NQO1, GCLc, GCLm, NF-κB, p-NF-κB, IκBα, and p-IκBα. Nrf2 knockout mice were used to assess the impact of CHE on the Nrf2 signaling pathway.

Results. The findings demonstrated that CHE significantly ameliorated oxidative damage, inflammatory response, and liver fibrosis in CCl_4-induced mice. CHE treatment increased Nrf2 expression and its target proteins, including HO-1 and GCLc, an effect not observed in Nrf2 knockout mice. In addition, CHE reduced NF-κB expression levels.

Conclusions. These results suggest that CHE can alleviate liver fibrosis in CCl4-induced mice by modulating NF-κB/IκBα and Nrf2 signaling pathways. These findings propose CHE as a potential novel anti-liver fibrosis drug.

Key words: Chelerythrine, CCl_4, Liver fibrosis, Nrf2/NF-κB signaling pathway

Corresponding Author: Xiaoli Wang, School of Pharmacy, Qiqihar Medical University, 333 BuKui Street, JianHua District, Qiqihar 161006, China. e-mail: ccwxl@126.com

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Introduction

Liver fibrosis denotes the pathological changes arising from the excessive accumulation of fibrotic collagen during liver tissue wound healing, holding a great chance of progressing to severe conditions such as liver failure, cirrhosis, and cancer (Dai et al., 2021). Damage or impairment of liver function profoundly impacts human health, with approximately two million global fatalities attributed to liver disease annually, posing a significant threat to public health and national economic sustainability (Devarbhavi et al., 2023). The mechanism of liver fibrosis involves diverse cellular and molecular processes, leading to structural alterations in liver tissue and fibrous tissue deposition. While early-stage liver fibrosis is considered reversible, its progression to advanced stages becomes irreversible, leading to cirrhosis and potentially liver cancer (Fan et al., 2018). Therefore, the key to treating liver fibrosis lies in preventative measures and reversal. The liver, playing a vital role in numerous physiological processes and the metabolism of exogenous substances, becomes susceptible to inflammation and fibrosis under prolonged exposure to a toxic environment (Lyu et al., 2020). Oxidative stress and inflammatory response are closely related to liver fibrosis. Oxidative stress triggers excessive production of reactive oxygen species, resulting in liver cell death and hepatic stellate cell (HSC) activation, the primary cell type driving liver fibrosis (Wang et al., 2018). Moreover, reactive oxygen species induce the release of inflammatory factors, intensifying liver injury. Consequently, the focal point of anti-liver fibrosis research revolves around inhibiting oxidative stress and inflammation (Zhu et al., 2021).

NF-κB, a pivotal transcription factor, regulates numerous vital biological processes. Part of the NF-κB/Rel protein family, NF-κB forms homologous or heterodimers. Initially recognized for its lymphoid tissue specificity, NF-κB typically resides in the cytoplasm as an inactive complex with inhibitory κB (IκB). Upon

exposure to proinflammatory agonists, IκB undergoes phosphorylation and degradation by IκB kinase, which facilitates NF-κB nuclear translocation. Subsequently, NF-κB translocates to the nucleus, binds with DNA, and induces target gene expression (Hoesel and Schmid, 2013; Giridharan and Srinivasan, 2018).

Studies indicate that NF-κB remains dormant in unstimulated cells, and when activated, it gradually translocates to the nucleus in response to intracellular oxidative stress. Oxidative stress triggers NF-κB activation in the cytoplasm, initiating nuclear translocation (Morgan and Liu, 2010). As a key regulator of liver fibrosis, NF-κB plays a pivotal role in HSC activation (Wang et al., 2012). Recent research on carbon tetrachloride (CCl_4)-induced liver fibrosis in mice revealed that NF-κB activation correlates with heightened HSC activation, while inhibiting NF-κB has been demonstrated to mitigate liver fibrosis in mice (Toriumi et al., 2013).

Nuclear factor-erythroid-related factor-2 (Nrf2) serves as the central regulator of gene expression mediated by antioxidant response elements (Park et al., 2015), safeguarding cells through the regulation of various downstream targets. Notably, Nrf2 exhibits significant antioxidative effects by modulating GCLc, GCLm, NQO1, and HO-1 (Li et al., 2017). Its heightened expression in the liver aids in detoxifying and eliminating harmful chemicals and their metabolites.

Liver fibrosis poses a substantial threat to personal health, emphasizing the paramount importance of preventive and therapeutic measures. However, the lack of specific drugs for liver fibrosis treatment underscores the urgency for effective interventions. Traditional Chinese medicine has demonstrated notable efficacy in liver fibrosis prevention and treatment, presenting a multifaceted approach with minimal side effects. From extensive research, chelerythrine (CHE) emerges as a potential Chinese herbal medicine component for liver fibrosis treatment (Liu et al., 2018; Li et al., 2021).

CHE (Fig. 1A) is an active component of *Chelidonium majus* L, known for its antibacterial, bacteriostatic, and anti-inflammatory effects (He et al., 2019). Our previous experiments showed that CHE could reverse CCl_4-induced liver fibrosis and TGFβ1-activated HSCs (Li et al., 2021). In addition, studies have shown that CHE inhibits pulmonary inflammatory immune responses and fibrosis, reducing the severity of COVID-19 symptoms by regulating Nrf2 and NF-κB signaling pathways (Valipour et al., 2021). While previous research hinted at the ability of CHE to inhibit pulmonary fibrosis via the Nrf2/antioxidant response element (ARE) signaling pathway (Liu et al., 2018), it remains unclear whether CHE regulates liver fibrosis through the Nrf2 and NF-κB signaling pathways.

Therefore, our study focuses on CCl_4-induced mice, aiming to elucidate the mechanism by which CHE influences liver fibrosis through the regulation of NF-κB and Nrf2 signaling pathways, employing immunohistochemistry, ELISA, western blot, and RT-PCR.

Materials and methods

Chemicals and reagents

The CCl_4 solution was obtained from Adamas (Shanghai, China), while olive oil was procured from Macklin (Shanghai, China). CHE standard powder (GR-134-180110), with a purity of 98%, was sourced from Nanjing Guangrun Bio-technology Co., Ltd.

Animals and experimental protocol

Healthy C57BL/6J mice, weighing 15-25 g, were provided unrestricted access to water and food in controlled conditions (12h light/dark cycle, 25°C ± 2°C). Wild-type mice (total of 50 mice) were categorized into the control group, CCl_4 model group, and low, medium, and high-dose groups of CHE (7mg/kg/d, ig, 14 mg/kg/d, ig, and 28 mg/kg/d, ig, respectively). Nrf2 knockout mice (total of 30 mice) were divided into the control group, CCl_4 model group, and high-dose group of CHE (28 mg/kg/d, ig), with 10 mice in each group. A 50% oil mixture of CCl_4 and pure peanut oil (5:5) was prepared. In the first week, both the CCl_4 model and CHE administration groups were intraperitoneally injected at a rate of 0.5 ml/kg CCl_4-olive oil (twice a week), followed by 50% intraperitoneal injections of a CCl_4-olive oil mixture at a rate of 1.2 ml/kg twice a week. Different doses of CHE were administered from the fourth to the eighth week, while the remaining

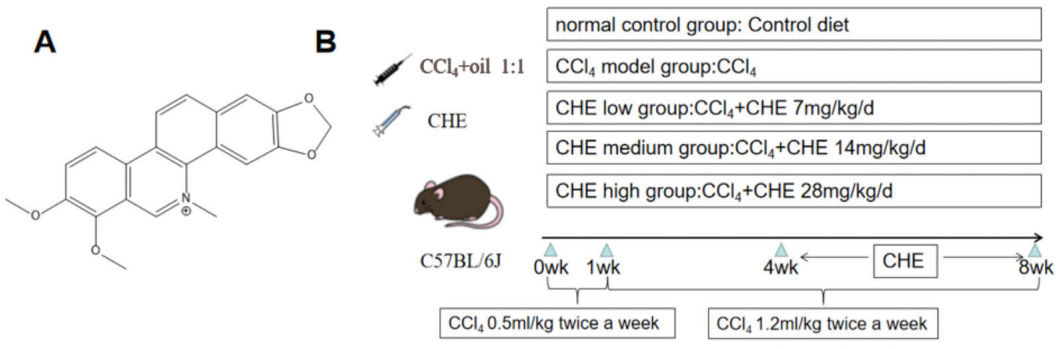

Fig. 1. Schematic diagram of the experimental design. **A.** Chemical structure of chelerythrine. **B.** Model diagram of drug administration in mice.

groups received solvent by gavage (Fig. 1B).

Upon completion of the study, the animals were treated according to the "Guidelines for the Care and Use of Experimental Animals", and the study was approved by the Animal Experimental Ethics Committee of Qiqihar Medical University (QMU-AECC-2021-13). Liver samples were preserved in formalin and deposited at −80°C. Immunohistochemistry, RT-PCR, ELISA, and western blotting were conducted to analyze the liver tissues.

AST/ALT determination

Blood from C57BL/6J mice was obtained through orbital blood sampling, and the ALT and AST levels were subsequently measured.

Real-time PCR

Total RNA was extracted from liver tissues using the TRIzol reagent (Invitrogen) following standard protocols. Subsequently, the PrimeScript RT reagent kit (#RR037A; Takara; Japan) was utilized to reverse-transcribe the RNA into cDNA using total RNA as the template. The RT-PCR system (Mx3000p™, Jitai Biotechnology Co., Ltd, Shanghai, China) facilitated the quantification of target mRNA expression, with a series of thermal cycles amplifying GAPDH as the reference gene. Quantitative analysis of the target molecule was achieved by comparing the Ct value of the target molecule with the standard curve. Primer sequences are listed in Table 1.

Inflammatory cytokines analysis

TNF-α, IL-1β, and IL-10 levels were individually assessed using ELISA kits (Shanghai Beyotime). The results for each group were obtained from six repeated experiments.

Histological and immunohistochemical analyses

Liver tissue was fixed in 10% formalin and subsequently embedded in paraffin. Liver tissue sections underwent staining with H&E, the Van Gieson method, Sirius red, and Masson trichrome for histological examination and liver fibrosis assessment. To further observe the degree of liver fibrosis, α-SMA, Col-I, and FN were examined by immunohistochemical methods. The primary antibody (α-SMA, Col-I, FN (Beyotime, Beijing, Chian)) was added to the paraffin sections of liver tissue, incubated for 24h, and after rinsing, the secondary antibody was added for 10 min, and then HRP Streptavidin was added for 10min. In addition, DAB staining (CWBIO, Taizhou, China) was used to identify and quantify the distribution of the protein of interest. Microscope imaging facilitated the selection of appropriate sections, and Image Pro Plus 6.0 software was used to quantify liver fibrosis. The proportion of connective tissue in the tissue slice was calculated.

Protein extraction and western blotting

Proteins were extracted from mouse liver tissue and quantified. After quantification, the samples underwent separation through SDS-PAGE electrophoresis and were transferred to an NC membrane. Following a 2h blocking period with 5% skim milk (Cat No: D 8340 100 g; Solarbio), the membrane was incubated overnight at 4°C with primary antibodies. Afterward, secondary antibodies (Goat Anti-Rabbit IgG HRP-conjugated, 1:1000, CWBIO, cat: cw0103) were applied for 2h at room temperature. Primary antibodies included Nrf2 (1:1000, Beyotime, AF7623), α-SMA (1:1000, CST), HO-1 (1:1000, Beyotime, AF1333), GCLc (1:1000, Beyotime, AF6969), GCLm (1:1000, Beyotime, AF6972), NQO1 (1:1000, Beyotime, AF7614), FN and Col-I (1:1000, Nanjing Jiancheng Bioengineering Institute), NF-κB (1:1000, Beyotime, AF0246), IκBα (1:1000, Beyotime, AF5204), p-NF-κB (1:1000, Beyotime, AF5875), p-IκBα (1:1000, Beyotime, AF5851), and GADPH (1:1000, CST).

Oxidative stress marker examination

Liver tissues underwent homogenization in PBS, followed by centrifugation at 3000 x g at 4°C for 10 min to obtain the supernatant. Subsequently, the contents of superoxide dismutase (SOD, A001-3), glutathione peroxidase (GSH-px, A006-2), and malondialdehyde (MDA, A003-1) were measured using the respective kits from Nanjing Jiancheng Bioengineering Institute.

Table 1. Primers used for RT-PCR detection of genes expression.

Gene	Species	Forward	Reverse
α-SMA	Mouse	CCCAGACATCAGGGAGTAATGG	TCTATCGGATACTTCAGCGTCA
collagenI	Mouse	TAAGGGTCCCCAATGGTGAGA	GGGTCCCTCGACTCCTACAT
FN	Mouse	GATTGGCGACAAGTGGAG	TAGGTGAACGGGAGGACA
Nrf2	Mouse	GATCCGCCAGCTACTCCCAGGTTG	CAGGGCAAGCGACTCATGGTCATC
HO-1	Mouse	CACGCATATACCCGCTACCT	CCAGAGTGTTCATTCGAGCA
NQO1	Mouse	CAAGTTTGGCCTCTCTGTGG	AAGCTGCGTCTAACTATATGT
GCLc	Mouse	ATGATAGAACACGGGAGGAGAG	TGATCCTAAAGCGATTGTTCTTC
GCLm	Mouse	GCCACCAGATTTGACTGCCTTT	CAGGGATGCTTTCTTGAAGAGCTT

Statistical analyses

The data were expressed as mean ± SD. Statistical analysis of the experimental data was conducted using SPPS 21.0 software, with intergroup comparisons tested using one-way ANOVA. $p<0.05$ was considered statistically significant in group differences.

Results

The protective impact of CHE on CCl_4-triggered liver injury

The study primarily investigated the protective impact of CHE against CCl_4-triggered liver injury in mice. Prolonged intraperitoneal administration of CCl_4 induced severe liver injury and fibrosis in the mice. As shown in Figure 2A, CCl_4 markedly elevated AST and ALT levels. Conversely, the CHE treatment group exhibited a significant decrease in AST and ALT levels compared with the model group. These findings indicate a protective effect of CHE against sustained CCl_4-induced liver damage in mice.

CHE ameliorates CCl_4-induced liver fibrosis in mice

The impact of CHE on CCl_4-induced liver fibrosis in mice was assessed by analyzing liver collagen content. Masson trichrome staining and modified Van Gieson staining in Figures 2B and 2D revealed a lack of fibrous connective tissue in the control group, contrasting with substantial fibrous connective tissue in the model group. However, in the CHE treatment group, an increase in dosage significantly reduced the presence of fibrous connective tissue. Sirius red staining exhibited no collagen deposition in the control group, while the model group showed extensive collagen deposition. In the CHE treatment group, an increase in dosage significantly decreased collagen deposition.

As displayed in Figures 2C and 2F, the liver tissue of the model group exhibited an elevated number of cells labeled with α-SMA, Col-I, and FN antibodies with the control group. However, in the CHE treatment group, the number of cells labeled with α-SMA, Col-I, and FN antibodies gradually decreased with increased drug dosage, indicating a weakening of liver fibrosis.

The results in Figures 2G-J demonstrated a substantial enhancement in the protein and mRNA levels of α-SMA, FN, and Col-I in CCl_4-treated mice. After CHE treatment, both mRNA and protein expression levels of α-SMA, FN, and Col-I markedly decreased. Combined with the above results, CHE effectively mitigates CCl_4-induced liver fibrosis *in vivo*.

CHE alleviates CCl_4-induced inflammation by inhibiting the NF-κB pathway

The impact of CHE on CCl_4-induced inflammatory cytokine levels was evaluated. As demonstrated in Figure 3A, CCl_4 significantly increased TNF-α and IL-1β levels, accompanied by a significant decrease in IL-10 levels. Conversely, TNF-α and IL-1β contents were lower, and IL-10 content was higher in the CHE treatment group compared with the model group.

As displayed in Figure 3B, normal mice exhibited clear liver lobule structures with normal liver tissue and no evident necrosis or degeneration. However, the model group showed hepatocyte fatty degeneration, numerous inflammatory cells, and fibrous connective tissues. In the CHE treatment group, an increase in dose corresponded with a reduction in inflammatory cells and a weakening of fibrosis.

Protein blot analysis of NF-κB, IκBα, p-NF-κB, and IκBα (Fig. 3C-E) indicated that CCl_4-treated mice exhibited increased levels of p-NF-κB and IκBα compared with the control group, which then decreased with escalating CHE concentration. These results demonstrate that CHE mitigates CCl_4-triggered inflammation by inhibiting the NF-κB signaling pathway.

CHE modulates CCl_4-induced liver oxidative stress in vivo

As shown in Figures 4A and 4B, after CCl_4 treatment, there was a significant increase in MDA levels compared with the control group, coupled with a notable decrease in GSH-px and SOD expression levels. Interestingly, these aberrations were effectively reversed in the CHE treatment group, with the content and activity approaching levels observed in the control group. This suggests that CHE can impede or reverse CCl_4-induced oxidative liver injury.

CHE inhibits liver fibrosis by activating the Nrf2 pathway

To investigate whether the Nrf2 signaling pathway is activated during CHE treatment in mice with liver fibrosis, western blot and RT-PCR were used to assess Nrf2 expression and that of its downstream antioxidant genes, such as GCLc, GCLm, HO-1, and NQO1 (Fig. 4C-4I). In the model group, GCLc and HO-1 expression was slightly increased compared with the control group. However, in the CHE treatment group, GCLc and HO-1 expression was distinctly enhanced compared with the model group. In addition, while the Nrf2 level mildly increased in CCl_4-treated mice with liver fibrosis, it significantly increased after CHE treatment. GCLm and NQO1 expression remained almost unchanged, suggesting that they may not be regulated by CHE. These findings indicate that CHE may inhibit CCl_4-triggered oxidative damage by mediating the Nrf2 signaling pathway.

Furthermore, to explore the role of Nrf2, Nrf2 knockout mice were utilized to assess related indexes using western blot, RT-PCR, and immunohistochemical staining. In Nrf2 knockout mice, GCLc and HO-1 mRNA and protein expressions showed minimal changes

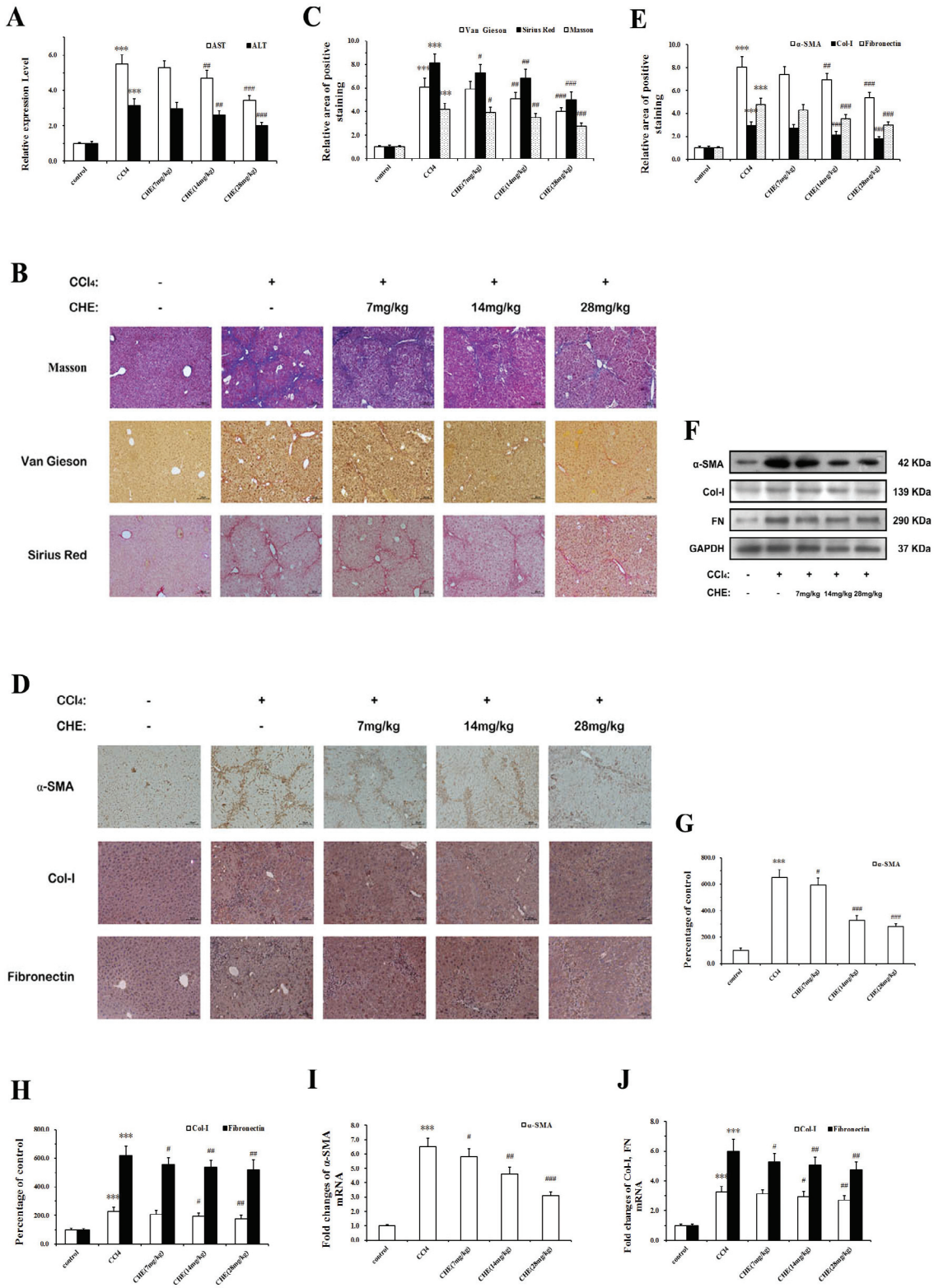

Fig. 2. CHE ameliorates CCl_4-induced liver injury and fibrosis in mice. **A.** Serum levels of AST and ALT. **B.** Representative images of Masson trichrome staining (×100), modified VG staining (× 100), and Sirius red staining (× 100) in five groups of liver tissues. **C.** Semi-quantitative analysis of liver fibrosis area in mice using different staining methods. **D.** Immunohistochemical staining results of α-SMA, Col-I, and FN in five groups of liver tissues (×100). **E.** Semi-quantitative analysis of liver fibrosis area in mice using immunohistochemical staining with α-SMA, Col-I and FN antibodies. **F.** Western blot analysis of α-SMA, Col-I, and FN. **G, H.** Relative protein expression levels of α-SMA, Col-I, and FN. **I, J.** mRNA expression levels of α-SMA, Col-I, and FN, n=6. Compared with the control group, $^{***}p<0.001$; Compared with the model group, $^{\#}p<0.05$, $^{\#\#}p<0.01$, and $^{\#\#\#}p<0.001$.

in the CHE administration group compared to the model group. However, compared with wild-type (WT) mice, GCLc and HO-1 mRNA and protein expression levels dramatically decreased in Nrf2 knockout mice after CHE treatment. (Fig. 5A-5E). In contrast to WT mice, in Nrf2-KO mice, the effect of CHE on inflammatory factors was significantly weakened (Fig. 5F-5H). α-SMA immunohistochemical staining (Fig. 5I-5J), western blot, and RT-PCR (α-SMA, Col-I) results revealed no obvious differences between the model and CHE treatment groups in Nrf2 knockout mice. Meanwhile, α-SMA and Col-I expression was higher in Nrf2 knockout mice than in WT mice, as shown in Figures 5K-5O. These results further emphasize that CHE partially exerts its anti-liver fibrosis role through the Nrf2 signaling pathway.

Discussion

Liver fibrosis, a prevalent chronic liver disease, was substantially ameliorated in CCl_4-induced mice through the intervention of CHE. This was achieved by restraining the NF-κB signaling pathway and promoting the Nrf2 pathway *in vivo*. Hepatocytes, pivotal for maintaining liver function, are vulnerable to damage, acting as the primary catalyst for liver fibrosis initiation and progression (Shrestha et al., 2016). Prolonged or repetitive injury induces hepatocyte death through apoptosis and necrosis, triggering inflammation and profibrotic pathways that escalate liver fibrosis development (Jangra et al., 2022). Notably, previous research, such as Zhang's study, underscored the ability of CHE to proliferation of SMMC-7721 cells (Zhang et al, 2011). Our current findings reinforce this, demonstrating that CHE not only enhances liver action, as evidenced by reduced serum AST and ALT levels and improved histopathological features, but also protects hepatocytes by enhancing their integrity. These results highlight the feasibility of CHE as a therapeutic drug for liver fibrosis, targeting key pathways to mitigate liver damage and promote recovery.

To research the influence of CHE on liver fibrosis, the liver collagen content was examined. Van Gieson, Sirius red, and Masson trichrome staining initially revealed severe CCl_4-induced liver fibrosis in mice,

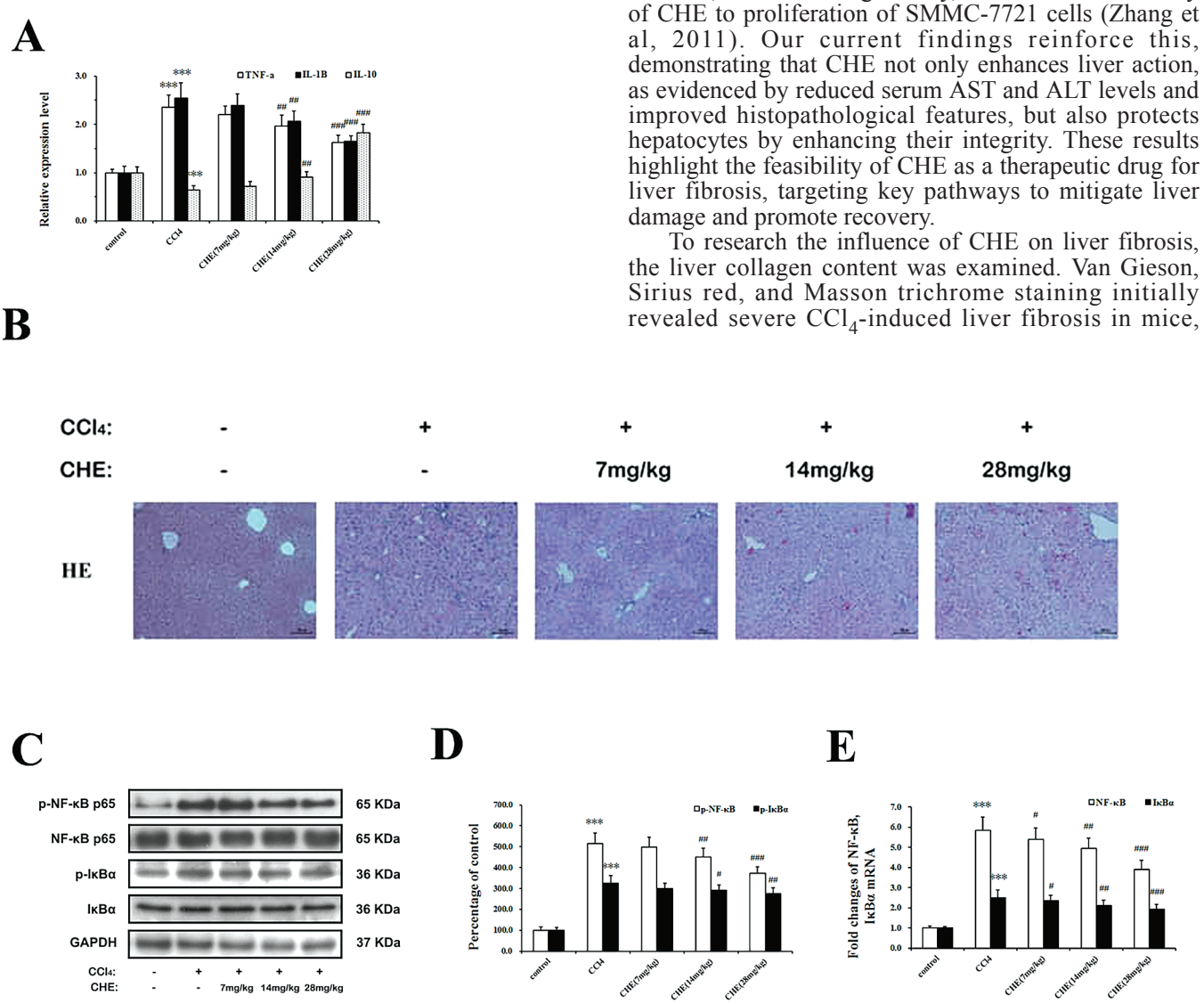

Fig. 3. CHE inhibits inflammation in CCl_4-induced mice through the NF-κB signaling pathway. **A.** Serum levels of TNF-α, IL-1β, and IL-10. **B.** HE staining results (× 100). **C.** Protein expression of NF-κB, IκBα, p-NF-κB, and IκBα. **D.** Relative protein levels of p-NF-κB and IκBα from (C). **E.** mRNA expression of NF-κB and IκBα, n = 6. ***p<0.001 vs. the control group; $^{\#}p$<0.05, $^{\#\#}p$<0.01, and $^{\#\#\#}p$<0.001 vs. the model group.

evidenced by extensive fibrosis and collagen deposition. Conversely, the fibrotic area in the CHE treatment group decreased with escalating CHE doses, signifying its potential to reduce CCl_4-induced liver fibrosis in mice. Subsequently, western blot and RT-PCR were employed to evaluate α-SMA, FN, and Col-I expression and mRNA levels. The results showed that CHE effectively inhibited CCl_4-induced collagen accumulation in mice, highlighting its capacity to curb HSC activation and alleviate liver fibrosis. These findings substantiate that CHE effectively improves CCl_4-induced liver fibrosis *in vivo*.

Liver fibrosis is closely linked to inflammatory reactions. Damaged livers generate abundant inflammatory factors, including TNF-α, IL-1β, and IL-10. The NF-κB signaling pathway plays a vital role in regulating these factors, influencing HSC activation and inhibiting HSC apoptosis. Inflammatory stimuli propel HSCs from

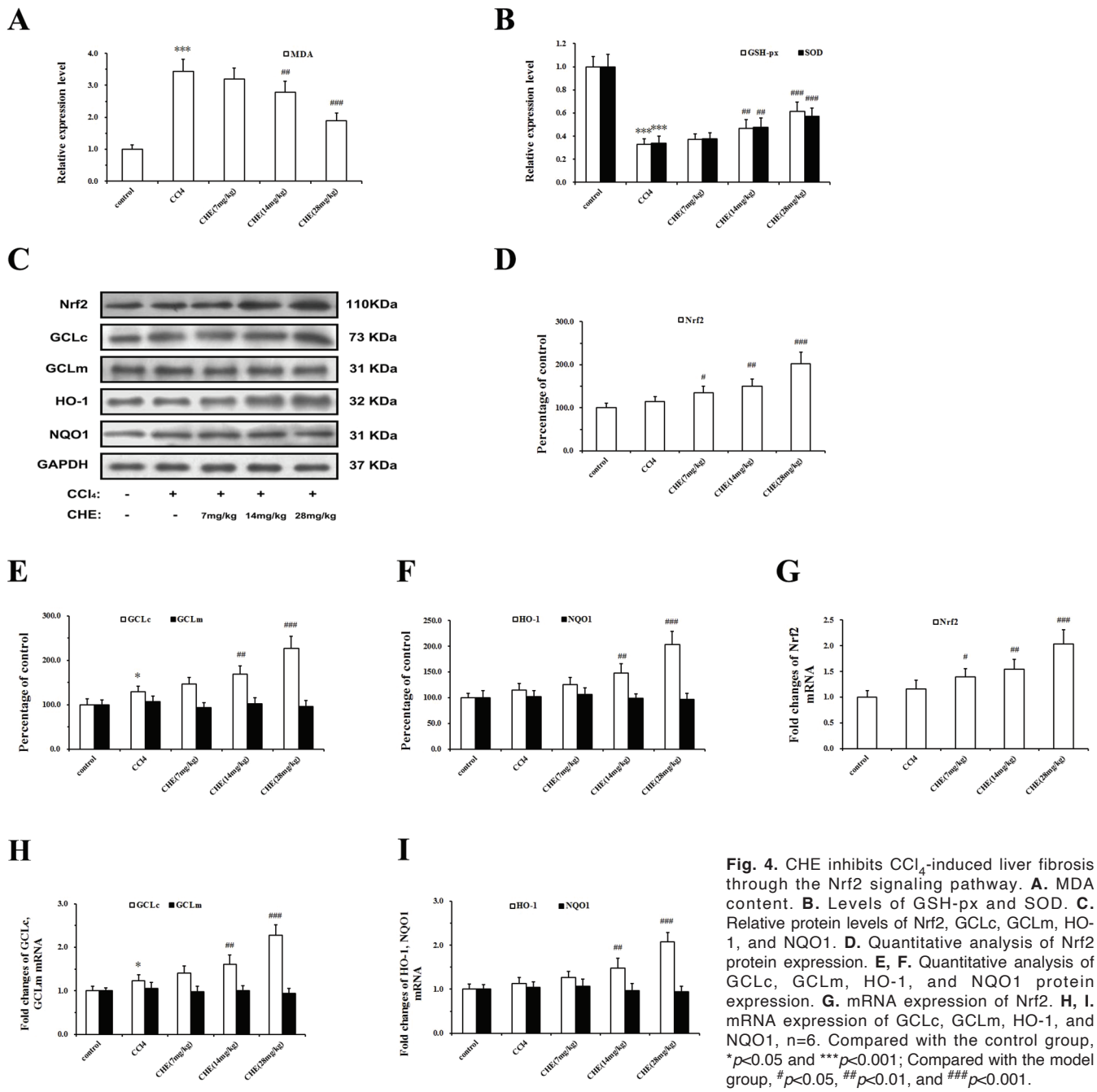

Fig. 4. CHE inhibits CCl_4-induced liver fibrosis through the Nrf2 signaling pathway. **A.** MDA content. **B.** Levels of GSH-px and SOD. **C.** Relative protein levels of Nrf2, GCLc, GCLm, HO-1, and NQO1. **D.** Quantitative analysis of Nrf2 protein expression. **E, F.** Quantitative analysis of GCLc, GCLm, HO-1, and NQO1 protein expression. **G.** mRNA expression of Nrf2. **H, I.** mRNA expression of GCLc, GCLm, HO-1, and NQO1, n=6. Compared with the control group, $^{*}p<0.05$ and $^{***}p<0.001$; Compared with the model group, $^{\#}p<0.05$, $^{\#\#}p<0.01$, and $^{\#\#\#}p<0.001$.

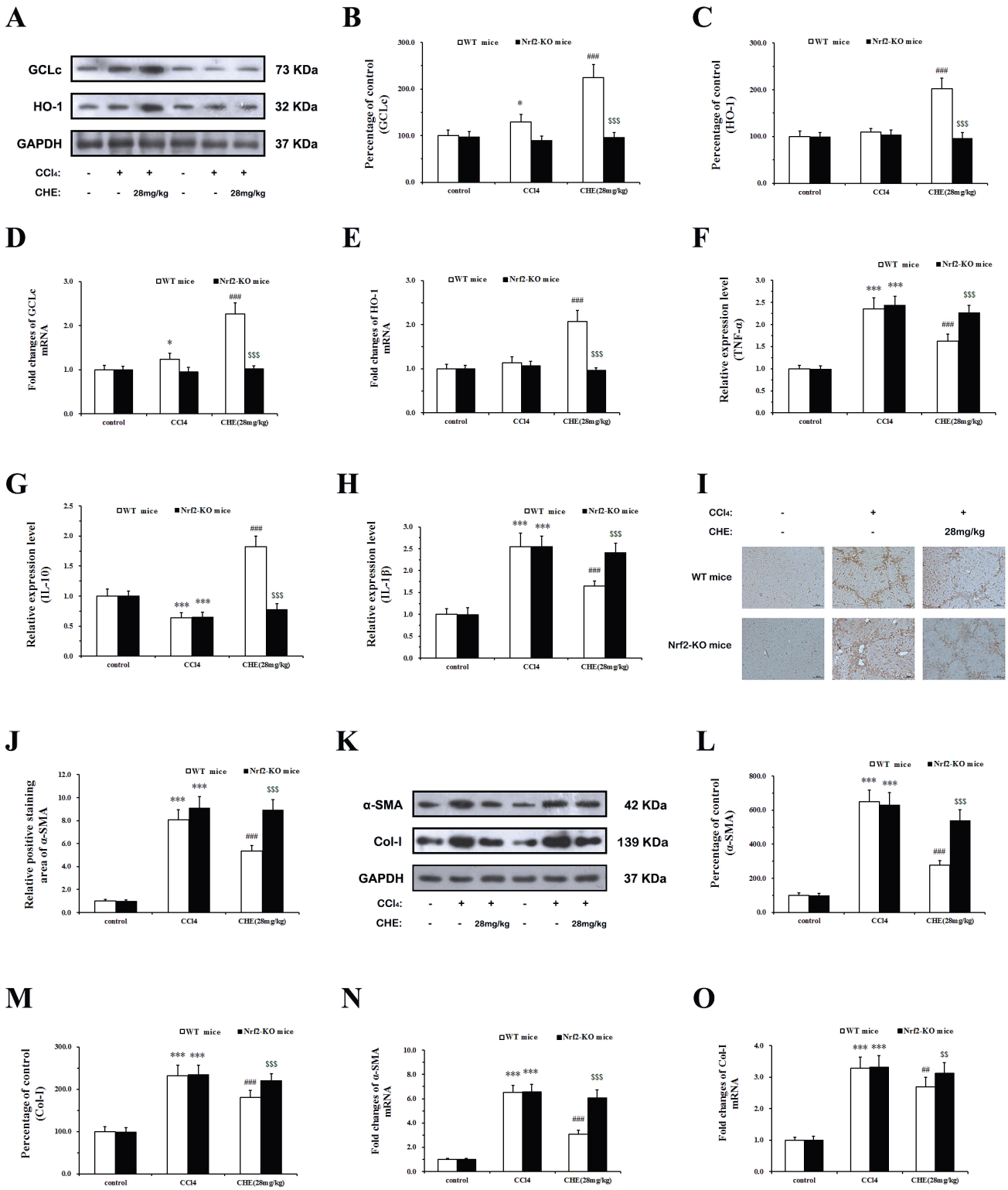

Fig. 5. Effects of CHE on liver fibrosis indexes in wild-type and Nrf2 knockout mice. **A-E.** Relative protein and mRNA expression of GCLc and HO-1 in wild-type and Nrf2 knockout mice. **F-H.** Inflammatory factors (TNF-α, IL-10, and IL-1β) were tested in WT and Nrf2-KO mice. **I, J.** α-SMA immunohistochemical staining results (×100) and semi-quantitative analysis of liver tissues in wild-type and Nrf2 knockout mice. **K-O.** Relative protein and mRNA expression of α-SMA and Col-I in wild-type and Nrf2 knockout mice, n=6. *$p<0.05$ and ***$p<0.001$ vs. the control group; $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ vs. the model group; $^{\$\$}p<0.01$, $^{\$\$\$}p<0.001$ vs. wild-type mice in the CHE group.

a quiescent to an active and proliferative state, fostering extracellular matrix (ECM) deposition and impairing liver immune function, thereby aggravating liver cell damage (Kamm and McCommis, 2022). Oxidative stress further activates the NF-κB pathway, stimulating HSCs and amplifying the production of TNF-α, intensifying inflammation and fibrosis (Mohamed et al., 2022). HSC apoptosis mitigates the inflammatory response, facilitating liver structure repair and fibrosis reversal. Studies have shown the efficacy of Trolline in improving liver fibrosis by suppressing the NF-κB signaling pathway and reducing the inflammatory response level (Bai et al., 2017). Gan Shen Fu Fang (GSFF) reportedly protects the liver and inhibits liver fibrosis by weakening TNF-α, IL-1β, and NF-κB synthesis (Du et al., 2020). In addition, strawberry extract plays an essential role in liver protection, thereby downregulating NF-κB and reducing inflammatory responses by weakening oxidative stress. In this paper, CCl_4-induced liver damage in mice treated with CHE exhibited a distinct decrease in TNF-α and IL-1β levels, accompanied by a substantial increase in IL-10 expression. To scrutinize the impact of CHE on the NF-κB signaling pathway, we assessed NF-κB transcription and translation levels. As anticipated, CHE markedly reduced p-NF-κB protein levels and NF-κB mRNA expression. Immunohistochemistry experiments corroborated these findings, showcasing a notable increase in NF-κB expression in the model group, followed by a dose-dependent decline with increasing CHE concentration. These results indicate that CHE effectively inhibits or alleviates liver fibrosis progression by suppressing the NF-κB signaling pathway and attenuating liver inflammatory reactions.

In the CCl_4-triggered liver injury mouse model, CCl_4 triggers liver inflammation, instigating the generation of reactive oxygen species, i.e., oxidative stress. This oxidative stress further activates HSCs, initiating their activation and proliferation as well as inducing liver fibrosis. Oxidative stress is recognized as a primary driver of hepatocyte death during liver fibrosis progression. It has been reported that interventions such as low-level laser therapy can mitigate liver fibrosis by inhibiting ECM production and deposition, oxidative damage, and inflammation and by upregulating PPARγ and Nrf2/HO-1 signal transduction (Mohamed et al., 2022). The literature also reports that allevieting oxidative stress can reverse liver injury or fibrosis (Aljobailly et al., 2020; Bai et al., 2023). In this context, CHE emerges as a key player in exerting antioxidant effects, as evidenced by its regulatory impact on serum MDA, SOD, and CAT levels (Wang et al., 2022). Our study corroborates these findings, revealing a substantial increase in MDA levels and a significant decrease in SOD and GSH-px activities in the CCl_4-treated group. Consistent with previous research, these findings report the efficacy of CHE in effectively inhibiting lipid peroxidation and oxidative stress, thereby protecting against CCl_4-induced hepatocyte injury.

The Nrf2/ARE pathway constitutes a cellular defense mechanism against diverse harmful stimuli, functioning as a crucial antioxidant system. Activation of the Nrf2 signaling pathway prompts Nrf2 to translocate from the cytoplasm to the nucleus, where it binds with ARE, inducing the expression of downstream antioxidant genes that safeguard against oxidative damage. Notably, ferulic acid has been verified to activate the Nrf2 signaling pathway, reducing oxidative stress and eliciting a prebiotic effect on host inflammatory responses (Zhang et al., 2022).

Recent studies underscore the therapeutic potential

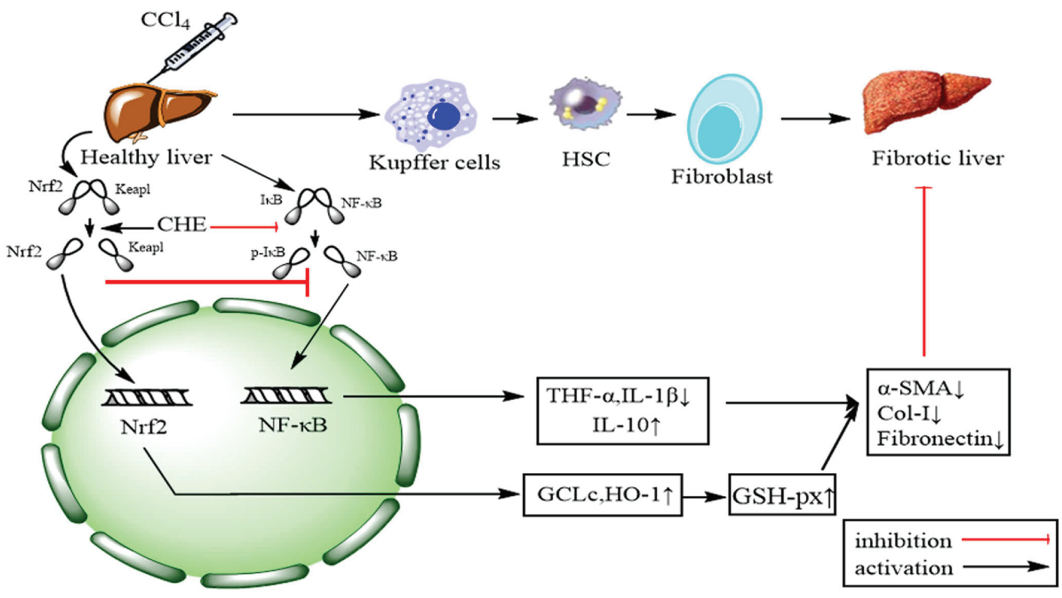

Fig. 6. Schematic diagram of the protective action of chelerythrine on CCl_4-induced liver fibrosis in mice.

of the Nrf2/ARE pathway in various liver diseases. Nrf2 gene-specific knockout mice exhibit increased susceptibility to liver fibrosis, exacerbating CCl_4-induced liver fibrosis, intensifying oxidative injury, and heightening inflammatory reactions (Lyu et al., 2020). This positions Nrf2 as a promising target for liver fibrosis treatment. Surprisingly, whether CHE can inhibit liver fibrosis by activating the Nrf2 pathway remains unexplored. In this study, CHE exhibited a remarkable increase in Nrf2 expression levels. In addition, the expression of Nrf2 target genes GCLc and HO-1 increased in a dose-dependent manner within the CHE treatment group. However, the expression levels of GCLm and NQO1 remained relatively constant. This suggests that CHE activates the expression of GCLc and HO-1, promoting GSH biosynthesis and exerting antioxidant effects. Importantly, this inhibition of liver fibrosis progression through the Nrf2 pathway highlights the antioxidant capabilities of CHE.

In the Nrf2 knockout group, the expression levels of GCLc and HO-1 remained consistent between the model and treatment groups. Although a slight decrease in α-SMA and Col-I levels was observed, it did not reach statistical significance, further reinforcing our findings. This change in α-SMA and Col-I expression suggests that CHE-induced inhibition of liver fibrosis is partly attributable to the antioxidant effects mediated by Nrf2. These findings show that CHE activates the Nrf2 signaling pathway, protecting against oxidative stress-mediated liver fibrosis in mice.

Moreover, despite distinct functional roles, Nrf2 and NF-κB exhibit certain interactions. Research suggests that Nrf2 can attenuate NF-κB expression, thereby inhibiting the release of pro-inflammatory mediators (Wardyn et al., 2015). Notably, Nrf2 has been reported to regulate NF-κB by reducing IκBα phosphorylation, leading to diminished nuclear accumulation of NF-κB. Cuadrado et al. highlighted the involvement of Nrf2 in the negative feedback loop of NF-κB regulation during the later stages, where the absence of Nrf2 left NF-κB without a controller to deactivate inflammatory signals (Cuadrado et al., 2014). The underlying mechanism likely involves Nrf2 inhibiting the activation and nuclear translocation of NF-κB, thereby diminishing the binding capacity of NF-κB to DNA and modulating the activity of associated signaling pathways. Our study also revealed that, in the absence of Nrf2, the effect of CHE on inflammatory factors was significantly weakened. This suggests that Nrf2 might partially influence NF-κB nuclear accumulation, disturbing inflammatory factor expression. However, it is crucial to note that the interaction between these pathways may vary across different cell types and pathological conditions. Therefore, further study is essential to delve into the regulatory mechanisms and physiological implications of their interplay.

Conclusion

In summary, our findings demonstrate that CHE effectively ameliorates liver fibrosis by activating the Nrf2 signaling pathway, inhibiting the NF-κB signaling pathway, and attenuating oxidative stress and inflammatory responses (Fig. 6). Therefore, under our experimental conditions, CHE emerges as a promising candidate for a novel drug in liver fibrosis treatment.

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