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ORIGINAL ARTICLE

Basic Study

Morin enhances hepatic Nrf2 expression in a liver fibrosis rat model

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Abstract

AIM

To investigate whether morin can reduce hepatic fibrosis by activating the NF-E2-related factor 2 (Nrf2) signaling pathway.

METHODS

Twenty male Sprague-Dawley rats were randomly divided into four groups: control group, morin group, carbon tetrachloride (CCl_4) group, and morin + CCl_4 group. Rats in both the CCl_4 and morin + CCl_4 groups were injected intraperitoneally with CCl_4 at a dose of 2 mL/kg twice a week. Rats in both the morin and morin + CCl_4 groups were treated orally with morin at a dose of 50 mg/kg twice a week. Control rats were treated with vehicle only twice a week. At the end-point of the 8 wk of the experimental period, serum AST, ALT, and ALP were measured, and the liver specimens

were obtained for pathological assessment. Real-time PCR and Western blot methods were used to analyze the expression of α-smooth muscle actin (α-SMA), collagen Ⅰ, collagen Ⅲ, Nrf2, heme oxygenase (HO-1), and quinone oxidoreductase 1 (NQO1) using frozen liver specimens.

RESULTS

Morin-treated rats in the morin + CCl_4 group had less hyperplasia of fiber tissue, minimal inflammatory cells, and less body weight loss with favorable liver enzyme measurements compared to rats treated with CCl_4 only. Additionally, morin-treated rats had significantly lower mRNA and protein expression of α-SMA, collagen Ⅰ, and collagen Ⅲ, but significantly higher mRNA and protein expression of Nrf2, HO-1, and NQO1 compared to rats treated with CCl_4 only ($P < 0.05$).

CONCLUSION

Morin could play a protective role by inducing the expression of Nrf2 and its downstream antioxidant factors (HO-1 and NQO1) and reducing the expression of α-SMA, collagen Ⅰ, and collagen Ⅲ in CCl_4-induced liver fibrosis rats.

Key words: Liver fibrosis; Rat; Morin; Nrf2

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Core tip: We constructed a liver fibrosis rat model with carbon tetrachloride (CCl_4). The Sprague-Dawley rats were randomly divided into four groups: control group, morin group, CCl_4 group, and morin + CCl_4 group. α-SMA, collagen Ⅰ, collagen Ⅲ, NF-E2-related factor 2 (Nrf2), heme oxygenase (HO-1), and quinone oxidoreductase 1 (NQO1) were analyzed by real-time PCR and Western blot methods using frozen liver specimens. We found that morin could reduce hepatic fibrosis by inducing the expression of Nrf2 and its downstream antioxidant factors in the CCl_4-induced rat liver fibrosis model.

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INTRODUCTION

Hepatic fibrosis refers to a series of pathogenic factors and pathological changes in the pathogenesis of a variety of liver diseases with liver extracellular matrix (ECM) metabolic abnormalities[1]. Previous studies have found that the development and progression of liver fibrosis are significantly related to oxidative stress in which a large number of free radicals lead to cell metabolic disorders and subsequent destruction of normal liver cells[2-5]. Although there is currently no effective therapy for curing liver fibrosis, previous studies showed that the pathological changes in liver fibrosis could be reversed[6,7].

Oxidative stress is closely related to the occurrence of liver disease[8]. A large number of studies have shown that oxidative stress may promote the activation of hepatic satellate cells (HSCs) and increase collagen production[9]. In the past decade, numerous studies proved that NF-E2-related factor 2 (Nrf2) plays a role as an important transcription factor in normal liver cells, and its activation could increase the expression of the downstream specific genes, such as the quinone oxidoreductase 1 (NQO1), heme oxygenase (HO-1), and glutathione, which play a role against oxidative stress[10,11]. Studies have shown that Nrf2 activation could resist oxidative stress caused by hepatic ischemia and injury, liver fibrosis, and drug-induced liver damage[12-15].

Flavonoids are rich in a variety of fruits, vegetables, and components of herbal-containing dietary agents and play an important role in preventing many kinds of diseases. Morin (3, 5, 7, 2′, 4′-pentahydroxyflavone) is a kind of flavonoid that consists of a yellowish pigment found in onion and apple[16], almond (P. guajava L.)[17], fig (*Chlorophora tinctoria*)[18], and other moraceae, including in food and herbal medicines[19] (Figure 1). It has been shown that morin possesses biological properties, including antioxidant[20,21], anti-inflammatory[22], anti-apoptosis[23,24], and anticancer[19] activities. Morin also protects various human cells, such as myoblasts[25], hepatocytes[26], and erythrocytes, against oxidative damages[27].

Carbon tetrachloride (CCl_4) intraperitoneal injection is a classical method for establishing an animal model of hepatic fibrosis, and the toxicity of CCl_4 leads to liver cell necrosis and mitochondrial damage along with aggravating oxidative stress. In addition, the abundant release of inflammatory and fibrogenic cytokines induced by CCl_4 could further augment the degree of hepatic fibrosis[28]. A previous study demonstrated that morin protected against acute liver damage[29] and ameliorated liver fibrosis[20] induced by CCl_4, where morin inhibited proliferation and induced apoptosis of activated HSCs by suppressing the Wnt/β-catenin and NF-kB signaling pathways. However, there is no molecular evidence of the effects of morin on the Nrf2 signaling pathway. To our knowledge, *in vivo* investigation of the effect of morin on the Nrf2 signaling pathway and Nrf2 expression in the CCl_4-induced liver fibrosis model has not been reported. The purpose of this study was to investigate whether morin could reduce hepatic fibrosis by inducing the expression of Nrf2 and its downstream antioxidant enzymes using pathology as a gold standard in a rat

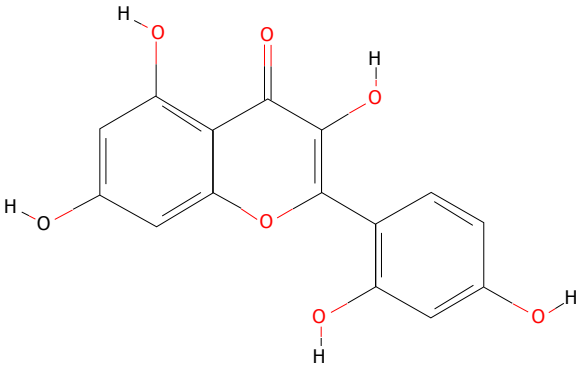

Figure 1 Chemical structure of morin. (https://pubchem.ncbi.nlm.nih.gov/compound/morin).

model of CCl_4-induced hepatic fibrosis.

MATERIALS AND METHODS

Chemicals and reagents

The chemical agents used in this study included CCl_4 and olive oil (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) as well as morin (Sigma Chemical Co., St Louis, MO, United States). Serum aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The antibodies against Nrf-2, HO-1, NQO1, collagen Ⅰ, collagen Ⅲ, and α-SMA were obtained from Proteintech Group Inc. (Chicago, IL, United States). All other reagents used were in the purest form available commercially.

Animals and experimental design

This study was performed in accordance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health of China (Guide for the Care and Use of Laboratory Animals, 1996) and was approved by the Animal Care and Use Committee of China Medical University. Twenty male Sprague-Dawley rats with an average body weight of 200-220 g (Changsheng Biotechnology Co., Ltd, Liaoning, China) were used in this study. All rats were fed a standard laboratory diet for a week at room temperature (20-22 ℃) with a light/dark cycle of 12 h. Then, the rats were randomly divided into four groups of five rats each, *i.e.*, control group, morin group, CCl_4 group, and morin + CCl_4 group. The control rats were treated with vehicle only (olive oil) equivalent to the treatment group. The rats in the morin group were treated with morin at a dose of 50 mg/kg (suspended in water as previously described[30]) by oral administration and 2 mL/kg of olive oil by intraperitoneal injection twice a week. The rats in the CCl_4 group were injected intraperitoneally with CCl_4 at a dose of 2 mL/kg [mixed with olive oil (40%, V/V)] twice a week. The rats in the morin + CCl_4 group were treated with the same doses of morin and CCl_4 *via* the same routes as the morin group and the CCl_4 group. Body weights of animals were recorded twice per week. After 8 wk of treatment, animals were kept fasting for 24 h. Under 10% chloral hydrate anesthesia, the following procedures were performed, including obtaining blood samples from the heart for biochemical tests and resecting the liver and spleen for histopathological analysis. Liver tissues were weighted and cut in 10 mm × 10 mm × 3 mm pieces. Half of the specimen was fixed in 10% formaldehyde for histopathology and the other half was immediately frozen in -80 ℃ for PCR and Western blot tests.

Biochemical analysis

The blood samples were centrifuged at 3000 *g* for 10 min at 20 ℃, and the serum was collected from the supernatant. The values of AST, ALT, and ALP were measured using commercial assay kits according to the manufacturer's protocols.

Histopathological assessment

Specimens of the liver were embedded in paraffin and cut into 5-μm-thick sections after 24 h of fixation. Then, the samples were stained with hematoxylin and eosin (HE). The degree of liver fibrosis was analyzed and determined by an experienced pathologist. The liver fibrosis was categorized into five degrees, *i.e.*, F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = portal fibrosis with rare septa, F3 = numerous septa without cirrhosis, and F4 = cirrhosis according to reference criteria[31].

Quantitative real-time PCR

Total cellular RNA was extracted from tissues using TRIzol (Invitrogen). Reverse transcription of 1 μg of RNA was done using RT regents (TAKARA) following the manufacturer's instructions. Quantitative real-time PCR was done using SYBR Green PCR master mix (Applied Biosystems) in a total volume of 20 μL on the 7900HT fast Real-time PCR system (Applied Biosystems) using the following cycling parameters: 50 ℃ for 2 min, 95 ℃ for 10 min, and 40 cycles of 95 ℃ for 15 s and 60 ℃ for 60 s. A dissociation procedure was performed to generate a melting curve for confirmation of amplification specificity. GAPDH was used as the reference gene. The relative levels of gene expression were represented as $\Delta Ct = Ct_{gene} - Ct_{reference}$, and the fold change of gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. Experiments were repeated in triplicate. The primer sequences are listed in Table 1.

Western blot analysis

Total proteins from tissues were extracted in lysis buffer (Pierce, United States) and quantified using the Bradford method. A total of 40 μg of protein were separated using 10% SDS-PAGE (80 V -120 V) and then electrophoretically transferred to a PVDF membrane

Table 1 Primer sequences

Name	Primer sequence
Rat *Collagen Ⅰ* for	5'-ACTGGTACATCAGCCCAAACCC-3'
Rat *Collagen Ⅰ* rev	5'-GGAATCCATCGGTCATGCTCT-3'
Rat *Collagen Ⅲ* for	5'-GAGACTCCCCATCATAGATATCGC-3'
Rat *Collagen Ⅲ* rev	5'-AGCAAACAGGGCCAATGTCC-3'
Rat α-*SMA* for	5'-GCTATGCTCTGCCTCATGCC-3'
Rat α-*SMA* rev	5'-CACGCTCAGCAGTAGTCACGAA-3'
Rat *Nrf2* for	5'-ACACAGCATAGCCCATCTCGT-3'
Rat *Nrf2* rev	5'-ACCAACCTGGATGAGCGACAC-3'
Rat *NQO1* for	5'-CCACGCAGAGAGGACATCATT-3'
Rat *NQO1* rev	5'-TTCGACCACCTCCCATCCTT-3'
Rat *HO-1* for	5'-CTTCCCGAGCATCGACAAC-3'
Rat *HO-1* rev	5'-CTGTCACCCTGTGCTTGACC-3'
Rat *Gapdh* for	5'-GCTGGTCATCAACGGGAAA-3'
Rat *Gapdh* rev	5'-CGCCAGTAGACTCCACGACAT-3'

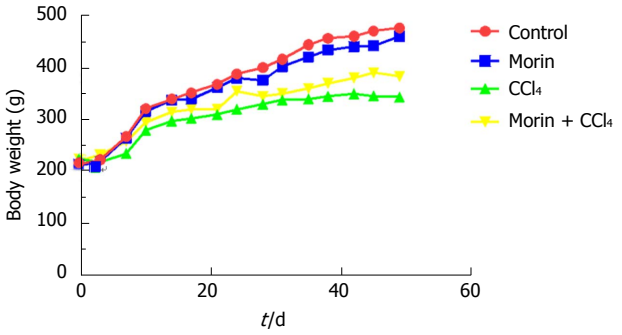

Figure 2 Changes in body weight among different groups. Body weight increased observably in the control and morin groups. The CCl_4 group had slow weight growth, but morin treatment was associated with increased body weight.

(80 V 100 min) (Millipore, Bedford, MA, United States). The membrane was blocked with 5% dry milk and incubated overnight at 4 ℃ with antibodies against HO-1 (1:800; Proteintech), NQO-1 (1:1000; Proteintech), Nrf2 (1:800; Proteintch), collagen Ⅰ (1:800, Proteintech), collagen Ⅲ (1:1000, Proteintech), α-SMA (1:1000, Proteintch), and GAPDH (1:4000, Proteintech). After washing, the membrane was incubated with a horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology) at 37 ℃ for 2 h. Protein bands were visualized by enhanced chemiluminescence (Pierce) and detected using BioImaging Systems (UVP, Upland, CA, United States). The relative protein levels were calculated based on GAPDH protein as a loading control. Western blot images were measured with ImageJ software, and the relative gray values of protein expression were analyzed semi-quantitatively.

Statistical analysis

The experimental data are expressed as the mean ± SD. Statistical analyses were performed using one-way analysis of variance (ANOVA) between groups, and unpaired comparisons were analyzed using the least significant difference method LSD *t*-test. A *P*-value of 0.05 or less was considered statistically significant. All analyses were conducted using SPSS version 17.0 (SPSS, Inc., Chicago, IL, United States) and Prism GraphPad software Version 6.01 (GraphPad Software Inc., San Diego, CA, United States).

RESULTS

General observation

A total of four rats died before the end-point of the study, including two in the CCl_4 group, one in the morin + CCl_4 group, and one in the morin group. All animals in the control group survived. Normal diet and daily activities were recorded in the control and morin groups, with body weight increasing rapidly. The CCl_4 group presented poor feeding and daily activities with slow weight growth. The morin + CCl_4 group presented milder symptoms compared with the CCl_4 group, with increased body weight, which was, however, lower than that in the control and morin groups (Figure 2).

Histological changes in the liver

The results of HE staining showed that the liver cells appeared with a normal morphology and regular lobular structure in the control and morin groups. The liver tissue of CCl_4 group rats showed inflammatory cell infiltration, with portal and central veins surrounded by fibrous tissue accompanied by fibrous septa. The lobular structure was fuzzy with clearly visible false lobules. In the morin + CCl_4 group, the liver tissue demonstrated less hyperplasia of fiber tissue and minimal inflammatory cells compared to the CCl_4 group (Figure 3A-D).

Liver-spleen ratio and liver weight index

Both the CCl_4 and morin + CCl_4 groups had increased liver-spleen ratio (LSR) and liver weight index (LWI) compared with the control and morin groups ($P < 0.05$). The LWI between the CCl_4 and morin + CCl_4 groups showed a significant difference ($P < 0.05$), while no statistically significant difference was found for LSR ($P > 0.05$) (Table 2).

Biochemical findings

The CCl_4 and morin + CCl_4 groups had increased ALT, AST, and ALP levels compared to the control and morin groups ($P < 0.05$), and CCl_4 without morin treatment dramatically increased ALT, AST, and ALP values (Table 3).

mRNA expression of α-SMA, collagen Ⅰ, collagen Ⅲ, Nrf2, HO-1, and NQO1

Compared with the control and morin groups, significantly higher mRNA expression of α-*SMA*, *collagen Ⅰ*, and *collagen Ⅲ* was observed in liver tissues in the CCl_4 and morin + CCl_4 groups ($P < 0.05$). However, the mRNA expression of these molecules in the morin + CCl_4 group was significantly less than that in the CCl_4 group ($P < 0.05$) (Figure 4).

In the CCl_4 and morin + CCl_4 groups, mRNA expression

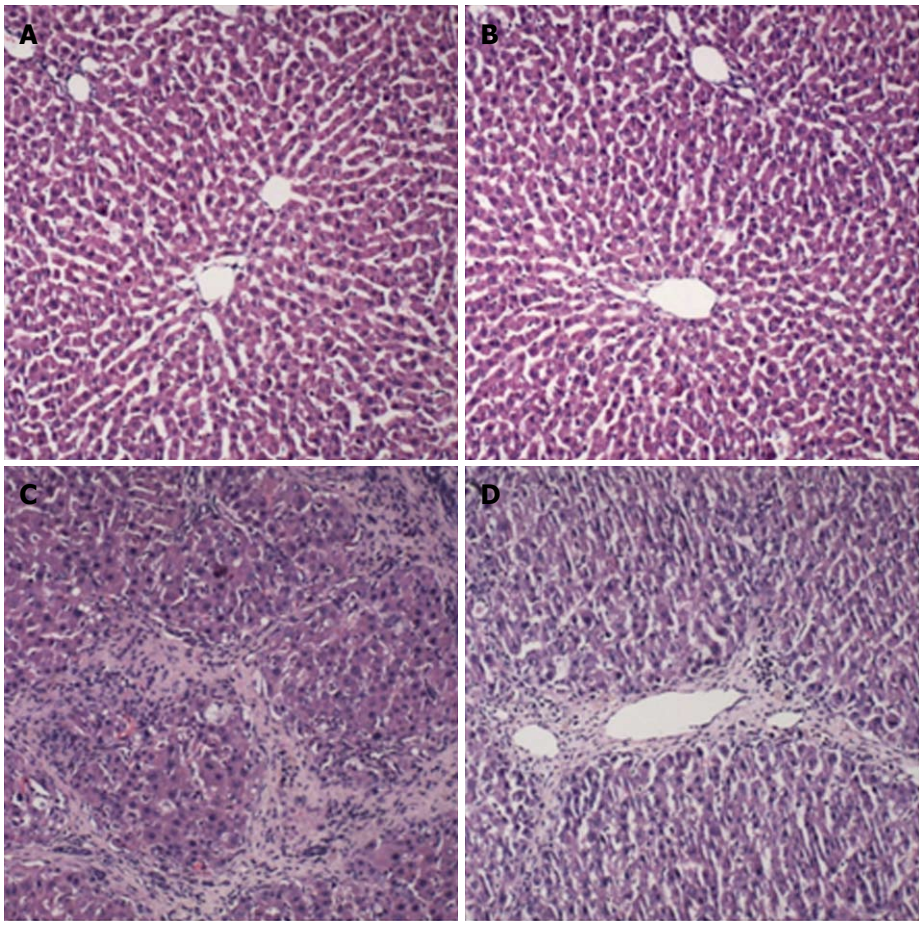

Figure 3 Histological changes of liver samples. A: Control group: treated with vehicle only; B: Morin group: treated with morin at a dose of 50 mg/kg twice a week; C: CCl_4 group: injected with CCl_4 at a dose of 2 mL/kg twice a week; D: Morin + CCl_4 group: treated with the same volume of morin and CCl_4 as the morin and CCl_4 groups. Liver tissues were stained with H&E (× 100).

Table 2 Comparison of liver-spleen ratio and liver weight index among different groups

	Control (n = 5)	Morin (n = 4)	CCl_4 (n = 3)	Morin + CCl_4 (n = 4)	F	P value
LSR	12.27 ± 1.92	12.67 ± 1.60	16.43 ± 1.37^{ac}	15.11 ± 1.99^{ac}	4.668	0.022
LWI%	2.78 ± 0.25	2.80 ± 0.27	4.77 ± 0.47^{ac}	4.17 ± 0.39^{ace}	32.345	< 0.001

^{a}P < 0.05 *vs* control group, ^{c}P < 0.05 *vs* morin group, ^{e}P < 0.05 *vs* CCl_4 group. Liver-spleen ratio (LSR): Liver wet weight/spleen wet weight; liver weight index (LWI): (Liver wet weight/body weight) × 100%.

Table 3 Serum parameters among different groups

	Control (n = 5)	Morin (n = 4)	CCl_4 (n = 3)	Morin + CCl4 (n = 4)	F	P value
ALT (IU/L)	101.75 ± 15.46	108.00 ± 48.72	493.33 ± 199.38^{ac}	291.50 ± 111.92^{ace}	11.403	0.001
AST (IU/L)	339.25 ± 72.59	257.80 ± 98.22	1027.67 ± 206.60^{ac}	585.50 ± 131.85^{ace}	26.280	< 0.001
ALP (IU/L)	137.75 ± 29.75	160.80 ± 40.90	377.67 ± 41.07^{ac}	266.50 ± 58.90^{ace}	22.093	< 0.001

^{a}P < 0.05 *vs* control group, ^{c}P < 0.05 *vs* morin group, ^{e}P < 0.05 *vs* CCl_4 group.

values of *NQO1*, *HO-1*, and *Nrf2* were significantly higher than those in the control and morin groups (P < 0.05), while these mRNA values of the morin + CCl_4 rats were significantly different compared to those of the CCl_4 group (P < 0.05) (Figure 5).

Protein expression of α-SMA, collagen Ⅰ, collagen Ⅲ, Nrf2, HO-1, and NQO1

Compared with the control and morin groups, high expression of protein of α-SMA, collagen Ⅰ, and collagen Ⅲ in liver tissues in the CCl_4 and morin +

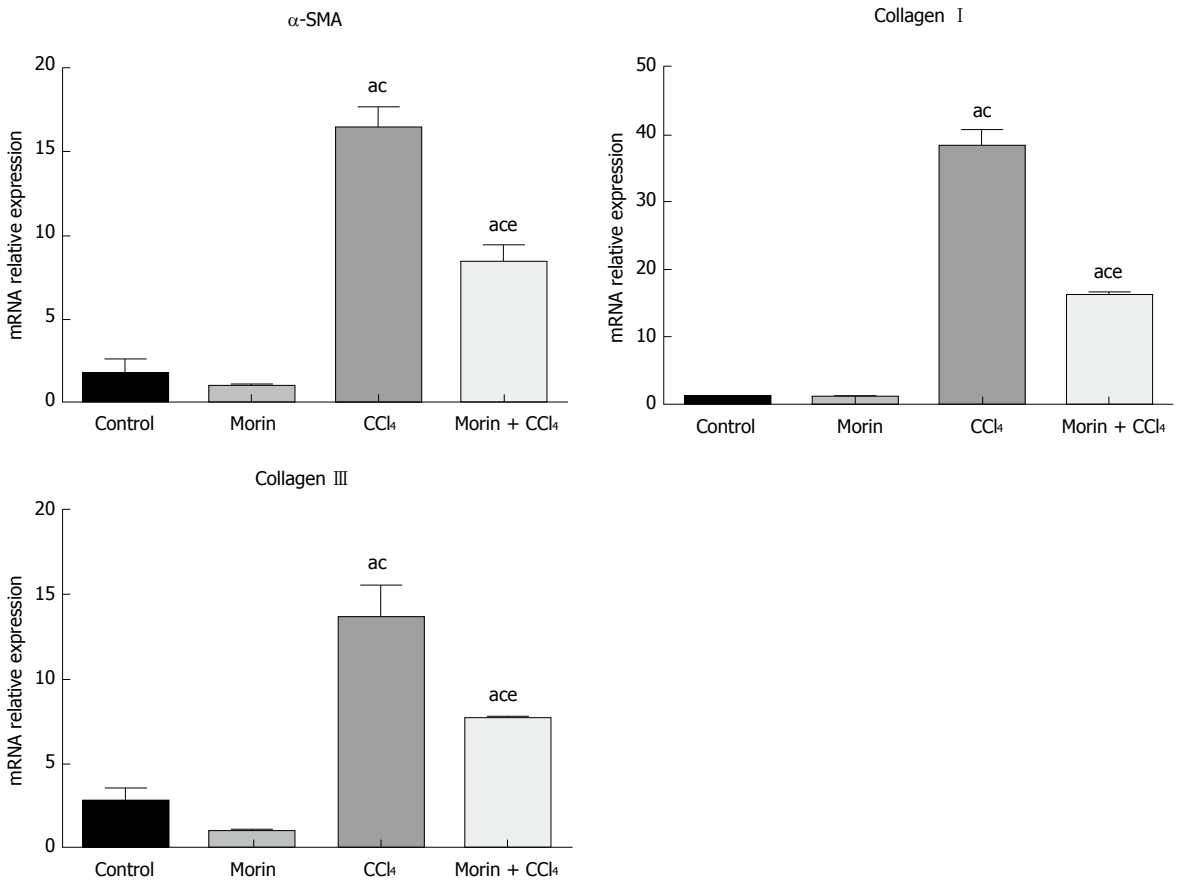

Figure 4 The mRNA expression of α-*SMA*, *collagen* Ⅰ, and *collagen* Ⅲ. $^{a}P < 0.05$ *vs* control group, $^{c}P < 0.05$ *vs* morin group, $^{e}P < 0.05$ *vs* CCl_4 group. In the control and morin groups, there was only minimal expression. The CCl_4 and morin + CCl_4 groups showed significantly increased expression ($P < 0.05$), while the expression levels in the morin + CCl_4 group were lower than those of the CCl_4 group ($P < 0.05$).

CCl_4 groups had a statistically significant difference ($P < 0.05$). However, the morin + CCl_4 group had less expression of these protein factors compared to the CCl_4 group ($P < 0.05$) (Figure 6).

In the CCl_4 and morin + CCl_4 groups, the protein expression of Nrf2, HO-1, and NQO1 was statistically higher than that in the control and morin groups ($P < 0.05$), while these protein factors of the morin + CCl_4 rats had more expression compared to the CCl_4 group ($P < 0.05$) (Figure 7).

DISCUSSION

Liver fibrosis is a process of continuous damage to the liver blood vessels and hepatic cells with nodule formation, which may develop into cirrhosis and cancerous lesions. Research of fibrosis at the cellular and molecular levels suggested that the progression of liver injury was closely related to oxidative stress and lipid peroxidation[32,33], leading to cell destruction and inducing hepatic fibrosis. HSCs can be activated by lipid peroxides acting as products of cell damage. After HSC activation, lipid droplets and vitamin A in the cytoplasm could be reduced or exhausted with α-SMA expression, accompanied by liver structural and functional changes resulting from redundant secretion of ECM[34]. However, it is possible to reverse liver fibrosis and early cirrhosis with effective interventions. Previous studies have shown that antioxidants have a protective effect by inhibiting the expression of α-SMA in HSC[35], thus, inhibition of oxidative stress in the liver may reduce and even reverse liver fibrosis[36].

Pathological features of liver fibrosis are reflected by fibrous tissue hyperplasia around the portal area and central vein and forming an interval of destruction of the lobular structure, accompanied by regenerative nodules and even early cirrhosis[37]. The pathological findings in this study showed that liver tissue in the CCl_4 group had liver cell necrosis, fibrous tissue hyperplasia, interval widening, and pseudolobuli replacing normal lobular architecture. In the morin + CCl_4 group, the liver tissue showed minimal cell necrosis with less interstitial collagen fibers and lobular structure damage compared with the CCl_4 group. Thus, morin could effectively protect the liver tissue by reducing inflammation and inhibiting collagen deposition and fiber hyperplasia.

There are various enzymes that take part in liver metabolism. The damaged liver cells by pathogenic factors will produce free enzymes that are released into the bloodstream[20]. Liver function and status could be assessed by assaying the contents of serum enzymes. Aminotransferases play an important role in

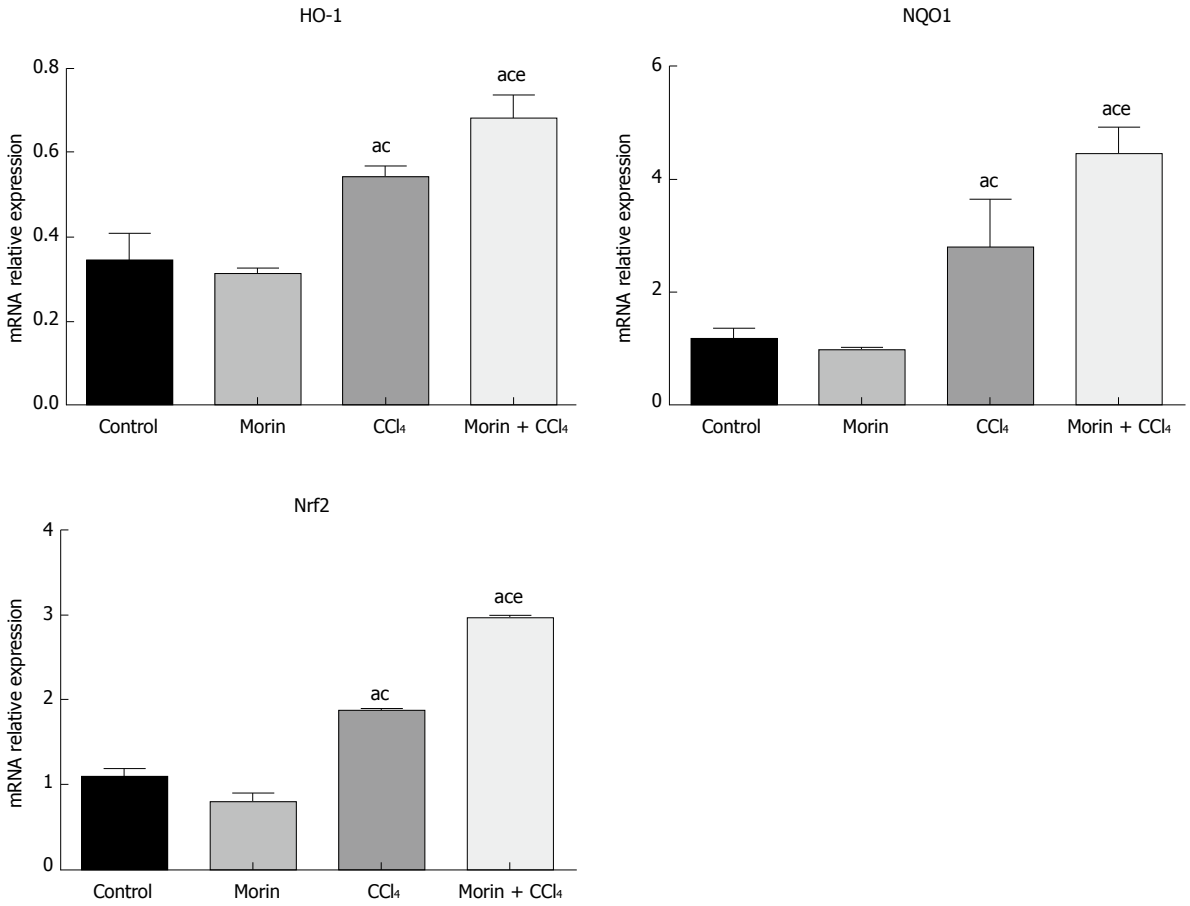

Figure 5 The mRNA expression of *HO-1*, *NQO1*, and *Nrf2*. $^{a}P < 0.05$ *vs* control group, $^{c}P < 0.05$ *vs* morin group, $^{e}P < 0.05$ *vs* CCl_4 group. The expression was increased obviously in the CCl_4 and morin + CCl_4 groups compared to the control and morin groups ($P < 0.05$). The expression levels in the morin + CCl_4 group were significantly higher than those of the CCl_4 group ($P < 0.05$).

hepatic metabolism. When the liver cells are damaged, the serum ALT and AST levels as well as ALP level will be increased[38]. In this study, in the CCl_4-induced liver fibrosis rat model, the values of serum ALT, AST, and ALP were reduced with morin administration, which implied that morin can reduce liver cell injury and thus prevent liver fibrosis. This also gives support for morin being able to condition the hepatocytes, protect against membrane frailty, and decrease the outflow of enzymes into circulation. These results are in accordance with previous studies that showed the ability of morin to inhibit hepatotoxicity[39,40].

The amount of collagen accounts for 5%-10% of the total protein in human liver tissue. If the liver injury leads to fibrosis, the collagen content in the liver protein will be significantly increased up to approximately 50%, becoming an important component of ECM[41] and ultimately leading to irreversible cirrhosis changes[42]. Liver fibrosis is a common histological change in liver disease, which is mainly manifested by excessive deposition of ECM, such as type Ⅰ and type Ⅲ collagen, and the expression of α-SMA[43]. At present, it is believed that the ECM actively participates in the occurrence and development of fibrosis, which has a great influence on HSC activation[44-46]. Both *in vitro* and *in vivo* experiments found that ECM synthesis was increased when liver tissue was damaged and further caused the activation of HSCs, which was based on the secretion of type Ⅰ and Ⅲ collagen[47-49], ultimately promoting the occurrence of liver fibrosis. In our study, using both real-time PCR and Western blot methods, it was found that the control and morin groups had only minimal expression of collagen Ⅰ, collagen Ⅲ, and α-SMA, which may represent normal physiological function of the liver, while their expression in the CCl_4 group was significantly increased and had great relevance to the severity of liver fibrosis. With morin intervention reducing the expression of collagen Ⅰ, collagen Ⅲ, and α-SMA, the degree of liver fibrosis was relieved, which was evidenced by liver histopathology and serum measurements. All these results suggested that the anti-fibrotic effect of morin may be related to the down-regulation of the expression of collagen Ⅰ, collagen Ⅲ, and α-SMA.

Nrf2 is a key nuclear transcription factor in the oxidative stress of various cells[50]. Under normal circumstances, Nrf2 and Keapl are in a binding state in the cytoplasm[51]; they will appear dissociated when oxidative stress is occurring[52] and combine with antioxidant components as dimers, which are

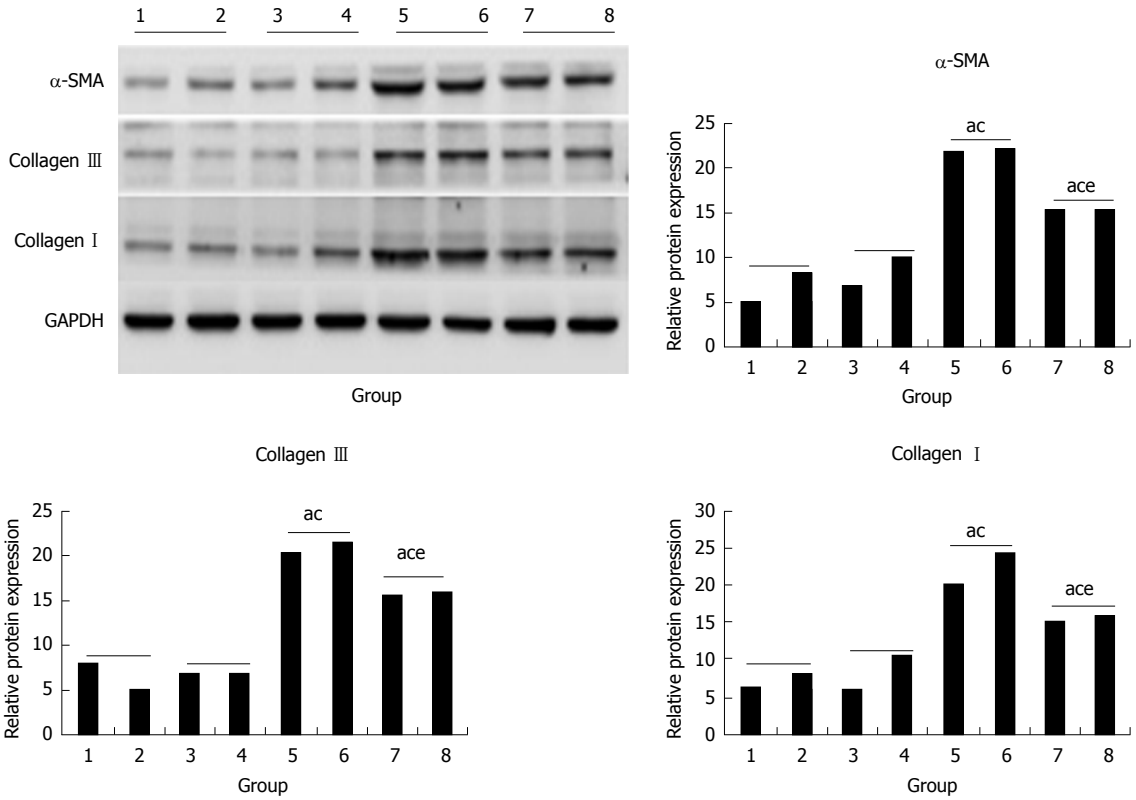

Figure 6 The protein expression of α-SMA, collagen Ⅲ, and collagen Ⅰ. (1, 2) control group, (3, 4) morin group, (5, 6) CCl_4 group, (7, 8) morin + CCl_4 group. $^aP < 0.05$ *vs* control group, $^cP < 0.05$ *vs* morin group, $^eP < 0.05$ *vs* CCl_4 group. The CCl_4 and morin + CCl_4 groups showed significantly increased expression compared to the control and morin groups ($P < 0.05$), and the expression levels in the morin + CCl_4 group were lower than those in the CCl_4 group ($P < 0.05$).

involved in the synthesis of antioxidase and phase Ⅱ detoxification enzymes and prevent the occurrence of liver fibrosis by improving the antioxidant capacity of the liver[53]. HO-1 and NQO-1 are well characterized Nrf2-dependent antioxidant defense genes. Studies have suggested that Nrf2 and its downstream antioxidant factors HO-1 and NQO1 may contribute to improvement of liver fibrosis[54]. It has been reported that morin could promote the nuclear translocation of Nrf2 in order to play its biological role and be used as an exogenous agonist of Nrf2[55]. In this study, a CCl_4 induced liver fibrosis model, along with morin as an intervention, was used to observe the expression of Nrf2 and its downstream products NQO1 and HO-1 in different groups. The results showed that the expression of Nrf2, NQO1, and HO-1 was slightly increased in the CCl_4 group compared with the control and morin groups ($P < 0.05$). This might be due to Nrf2 activation acting as a cellular adaptive response against CCl_4-induced toxicity. Nrf2 activation was initiated as soon as the subjects were challenged by CCl_4-induced oxidative stress. However, it was unable to completely overcome the toxicity, while the adaptively stimulated Nrf2 might alleviate or delay the deleterious effects of CCl_4. The expression of Nrf2 and its downstream products NQO1 and HO-1 was evidently increased in the morin-treated group, indicating that morin administration could enhance this effect. Additionally, this supports morin playing an important role in the prevention and treatment of liver fibrosis *via* the Nrf2 pathway.

This study has several limitations. First, the sample size was small, which easily led to individual differences and statistical error between the groups. Second, the anti-fibrotic mechanism of morin may be related to activation of the Nrf2 antioxidant pathway and expression of its downstream antioxidases. Further experiments are needed to confirm the specific mechanism of the morin intervention.

In summary, our current study showed that morin could play a protective role by inducing the expression of Nrf2 and its downstream antioxidant factors (HO-1 and NQO1) and reducing the expression of α-SMA, collagen Ⅰ, and collagen Ⅲ in a rat model of CCl_4-induced hepatic fibrosis. Although further studies are required, our study demonstrated that morin could effectively alleviate chronic liver damage by activation of the Nrf2 pathway.

ARTICLE HIGHLIGHTS

Research background

Previous studies have shown that the pathological changes of liver fibrosis, which refer to a series of pathogenic factors and pathological changes in the pathogenesis of a variety of liver diseases, could be reversed. In the past decade, numerous studies demonstrated that NF-E2-related factor 2 (Nrf2) as a transcription factor plays as an important role against oxidative stress in normal liver cells. Morin possesses biological properties, including antioxidant, anti-inflammatory, anti-apoptosis, and anticancer activities. To our knowledge,

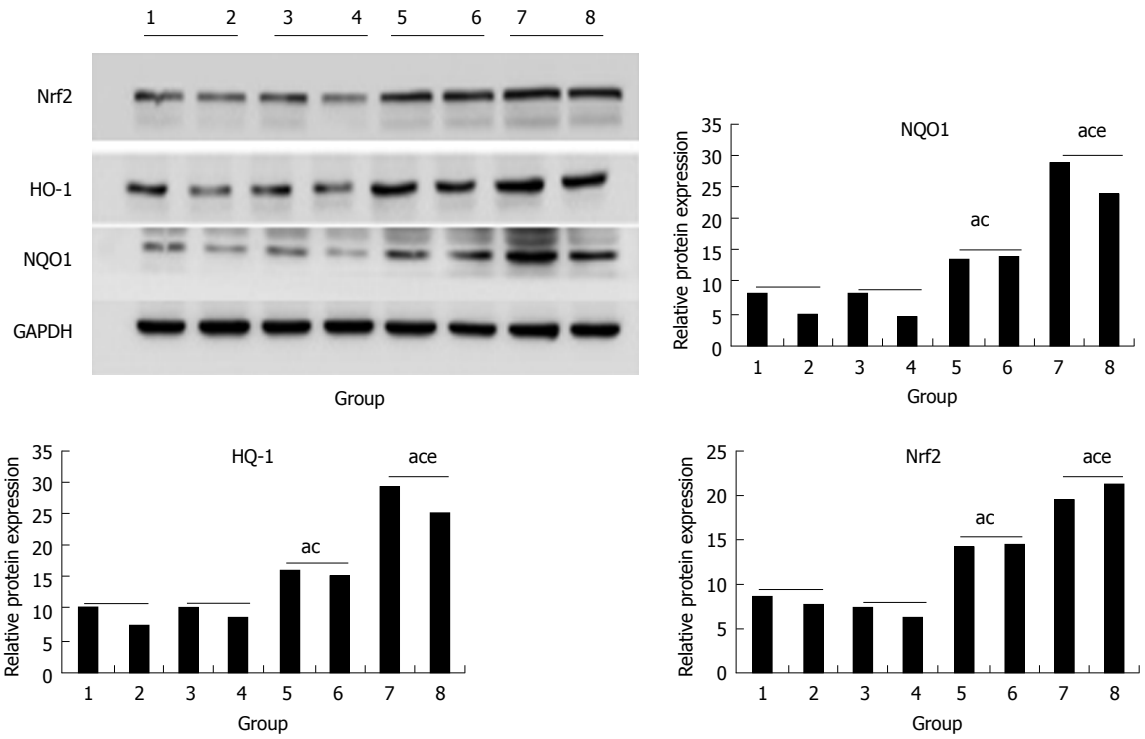

Figure 7 The protein expression of Nrf2, HO-1, and NQO1. (1, 2) control group, (3, 4) morin group, (5, 6) CCl_4 group, (7, 8) morin + CCl_4 group. $^{a}P < 0.05$ *vs* control group, $^{c}P < 0.05$ *vs* morin group, $^{e}P < 0.05$ *vs* CCl_4 group. In the CCl_4 and morin + CCl_4 groups, the protein expression was increased compared to the control and morin groups ($P < 0.05$); the morin + CCl_4 group had a more significant change compared to the CCl_4 group ($P < 0.05$).

in vivo investigation of the effect of morin on the Nrf2 signaling pathway and Nrf2 expression in a CCl_4-induced liver fibrosis model has not been reported previously.

Research motivation

Previous studies demonstrated that morin protected acute liver damage and ameliorated liver fibrosis induced by CCl_4, and morin inhibited proliferation and induced apoptosis of activated hepatic satellate cells by suppressing the Wnt/β-catenin and the NF-kB signaling pathways. However, there is no molecular evidence about the effects of morin on the Nrf2 signaling pathway.

Research objectives

The purpose of this study was to investigate whether morin can reduce hepatic fibrosis by inducing the expression of Nrf2 and its downstream antioxidant enzymes in a rat model of CCl_4-induced hepatic fibrosis.

Research methods

Twenty male Sprague-Dawley rats were randomly divided into four groups: control group, morin group, carbon tetrachloride (CCl_4) group, and morin + CCl_4 group. At the end-point of the experimental period, serum AST, ALT, and ALP were measured, and the liver specimens were obtained for pathological assessment. α-SMA, collagen Ⅰ, collagen Ⅲ, NF-E2-related factor 2 (Nrf2), heme oxygenase (HO-1), and quinone oxidoreductase 1 (NQO1) were analyzed by real-time PCR and Western blot methods using frozen liver specimens.

Research results

Rats in the morin + CCl_4 group had less hyperplasia of fiber tissues, minimal inflammatory cells, and less body weight loss with favorable liver enzyme measurements compared to rats treated with CCl_4 only. Additionally, morin-treated rats had significantly lower mRNA and protein expression of α-SMA, collagen Ⅰ, and collagen Ⅲ, but significantly higher mRNA and protein expression of Nrf2, HO-1, and NQO1 compared to rats treated with CCl_4 only ($P < 0.05$).

Research conclusions

Our study showed that morin could play a protective role by inducing the expression of Nrf2 and its downstream antioxidant factors (HO-1 and NQO1) and reducing the expression of α-SMA, collagen Ⅰ, and collagen Ⅲ in a rat model of CCl_4-induced hepatic fibrosis.

Research perspectives

Although further studies are required, out study demonstrated that morin could effectively alleviate chronic liver damage by activation of the Nrf2 pathway.

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