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Apigenin ameliorates CCl₄-induced hepatic fibrosis by inhibiting hepatic stellate cell activation *via* PI3K/AKT/GSK3 β pathway in mice

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ABSTRACT

Objective: To investigate the effect of apigenin on carbon tetrachloride (CCl₄)-induced liver fibrosis and elucidate the underlying mechanisms.

Methods: A mouse model of CCl₄-induced liver fibrosis was used to evaluate the effects of apigenin. Liver function was assessed using biochemical tests, and inflammation-associated markers, including interleukin-1 beta (IL-1 β), IL-6, IL-10, and tumor necrosis factor-alpha (TNF- α), were determined by enzyme-linked immunosorbent assay (ELISA). H&E staining, Sirius Red staining, and collagen immunohistochemistry were also conducted. In addition, antioxidant enzyme activity and the underlying mechanisms of hepatoprotective effects of apigenin were examined.

Results: CCl₄ administration induced hepatic stellate cell activation and liver fibrogenesis in mice. Apigenin treatment markedly decreased liver injury markers, inflammation, oxidative stress, and collagen deposition, mitigating CCl₄-induced liver fibrosis. Furthermore, it significantly suppressed the activation of the phosphoinositide 3-kinase/protein kinase B/glycogen synthase kinase 3 beta (PI3K/AKT/GSK3 β) pathway by reducing the ratios of p-PI3K/PI3K, p-AKT/AKT, and p-GSK3 β /GSK3 β in liver tissue.

Conclusions: Apigenin ameliorates CCl₄-induced liver fibrosis in mice, likely through the inhibition of the PI3K/AKT/GSK3 β signaling pathway. These findings suggest that apigenin may have therapeutic potential for treating liver fibrosis.

KEYWORDS: Liver fibrosis; CCl₄; Hepatic stellate cell; PI3K/AKT/GSK3 β ; Apigenin

1. Introduction

Cirrhosis, a significant health threat, is the common end stage of various chronic progressive liver diseases, including viral hepatitis and fatty liver. It represents the culmination of ongoing liver damage. Liver fibrosis, the critical precursor to cirrhosis, is a key stage in the progression from liver injury to fully developed cirrhosis caused by chronic liver diseases. This inherently complex process involves hepatocyte damage, inflammatory cell infiltration, mesenchymal cell activation, and accumulation of the extracellular matrix (ECM), along with other cellular and biochemical disturbances[1].

The activation of hepatic stellate cells (HSCs) plays a key role in

Summary

Question: Does apigenin alleviate carbon tetrachloride (CCl₄)-induced liver fibrosis, and what are its underlying mechanisms?

Findings: Apigenin significantly mitigated CCl₄-induced liver injury by reducing inflammation and oxidative stress, as well as inhibiting the PI3K/AKT/GSK3 β signaling pathway.

Meaning: Apigenin demonstrates potential therapeutic effects on liver fibrosis.

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fibrosis development. In their normal state, HSCs are quiescent, exhibiting minimal growth and replication, primarily storing vitamin A and producing scant ECM[2]. However, liver injury triggers an inflammatory response, stimulating various liver cells and leading to the secretion of pro-fibrotic factors. These factors induce HSC activation, characterized by a transition to a myofibroblastic phenotype, increased proliferation, and ECM deposition, including substances such as α -smooth muscle actin (α -SMA), collagen types I and III[3,4]. Concurrently, a decline in matrix metalloproteinase (MMP) activity, an increase in tissue inhibitor of metalloproteinase (TIMP) levels, and a slowdown in collagen breakdown contribute to excessive ECM buildup, exacerbating fibrosis[5]. Moreover, activated HSCs release cytokines that perpetuate their activation and the fibrotic process. Thus, targeting HSC activation is crucial in treating liver fibrosis.

Apigenin is a natural flavonoid found in various plants and foods, known for its wide spectrum of pharmacological effects[6]. Research indicates that apigenin can reduce renal failure and fibrosis in rats by downregulating the expression of TGF- β 1, fibronectin, and type IV collagen[7]. It also appears to lessen hepatic fibrosis by inhibiting hepatic HSC activation and autophagy *via* the transforming growth factor beta 1/SMAD family member 3 (TGF- β 1/Smad3) and p38 mitogen-activated protein kinase/peroxisome proliferator-activated receptor alpha (p38/PPAR α) pathways[8]. Furthermore, apigenin can hinder the growth and specialization of renal fibroblasts, suggesting its potential in treating renal fibrosis[9]. Recent research also shows that apigenin can effectively suppress HSC activation[10], but the underlying mechanisms remain unclear.

Our previous results of network pharmacology and molecular docking indicated a strong binding affinity between apigenin and targets like protein kinase B (AKT) and MMP-9[11]. Therefore, we hypothesize that apigenin may mitigate HSC activation through the phosphoinositide 3-kinase/AKT/glycogen synthase kinase 3 beta (PI3K/AKT/GSK3 β) pathway. In this study, the mechanisms of hepatoprotective action of apigenin were investigated in an animal model of liver fibrosis.

2. Materials and methods

2.1. Reagents and materials

Apigenin (purity $\geq 98\%$) was purchased from Chengdu Must Bio-Technology. Additional chemicals such as carbon tetrachloride (CCl₄) and olive oil were bought from Sinopharm (Shanghai, China). Biochemical assay reagents, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin (TBil), hydroxyproline, and superoxide dismutase (SOD) were obtained from Jiancheng Bioengineering

Institute (Nanjing, China). Kits for malondialdehyde (MDA), catalase, total antioxidant capacity, IL-1 β , and TNF- α were obtained from Servicebio (Wuhan, China). Radioimmunoprecipitation assay (RIPA) lysate, bicinchoninic acid assay kit, and enhanced chemiluminescence (ECL) solution were provided by Solarbio LIFE SCIENCES (Beijing, China). Antibodies such as α -SMA (19245S), vimentin (5741S), desmin (5332S), and PI3K (4257F) were supplied by Cell Signaling Technology (Massachusetts, USA). p-PI3K was sourced from Abcam (278545, Cambridge, USA). AKT (60203-2-Ig), p-AKT (28731-1-AP), GSK3 β (22104-1-AP), and p-GSK3 β (67558-1-Ig) were supplied by Proteintech Group Inc. (Wuhan, China).

2.2. Animals and treatment

Following acclimatization for one week, seventy-two SPF male C57BL/6 mice [aged 4–6 weeks, weighing (20 $\pm$ 2) g] from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) were categorized into six groups: the normal group, the model group, and three apigenin treatment groups (high, medium, and low), as well as colchicine group, with 12 mice per group. Apart from the normal group, all mice received intraperitoneal injections of a 10% CCl₄-olive oil solution (2 mL/kg), twice a week for four weeks. Starting in the fifth week, two hours post-injection of CCl₄, the apigenin treatment groups were orally administered with apigenin at varying doses of 80 mg/kg, 40 mg/kg, and 20 mg/kg once daily[8]. The control group and the model group were given an equal volume of olive oil, while the colchicine group received colchicine once daily. This treatment regimen spanned eight weeks.

Upon completion of the treatment period, mice were anesthetized with 3% pentobarbital sodium. Blood samples were collected from the inferior vena cava, centrifuged at 3 500 rpm for 15 min to isolate serum, and stored at -80°C . After blood collection, the liver was swiftly excised. Sections of liver tissue were utilized for routine H&E staining and immunohistochemical analysis, while the remaining tissue was promptly frozen in liquid nitrogen for subsequent assays. The liver tissue was then mixed with 0.9% saline at a tissue-to-saline ratio of 1:9 (mg/ μL). The mixture was homogenized in an ice-water bath to prepare a 10% homogenate solution. The solution was centrifuged at 2 500–3 000 rpm for 10 min, and the supernatant was collected for biochemical and ELISA measurement.

2.3. Biochemical analysis

Serum samples were separated from blood *via* centrifugation at 4 000 $\times g$ at 4 $^{\circ}\text{C}$ for 10 min. Serum levels of ALT, AST, ALP, TBil, and hydroxyproline were measured using commercialized test kits.

The activities of SOD and catalase, total antioxidant capacity, and

MDA levels in the liver homogenate were measured according to the biochemical kit instructions.

2.4. ELISA

The contents of IL-1 β , IL-6, TNF- α , and IL-10 in the liver homogenate were measured according to the ELISA kit instructions.

2.5. Histopathological examination

Liver tissue was fixed in neutral formalin, embedded in paraffin, and sectioned into 5 μ m thick slices. After deparaffinization and hydration, H&E and Sirius Red staining were performed. The pathological morphology of the liver tissue and collagen fiber deposition were observed under a microscope (Nikon, Tokyo, Japan).

Three different fields of view from each section were selected to observe the degree of fibrosis. The Scheuer staging system was applied to rate the extent of liver damage. The higher the rating, the more severe the liver fibrosis[12,13]. The grading is as follows: Grade 0: No fibrosis. Grade 1: Portal area shows expanded fibrosis, localized perisinusoidal and intralobular fibrosis. Grade 2: Periportal fibrosis, formation of fibrous septa, with lobular structure preserved. Grade 3: Extensive formation of fibrous septa with lobular structure disorder but without cirrhosis. Grade 4: Early cirrhosis.

2.6. Immunohistochemical analysis

Immunohistochemical methods were used to detect the levels of α -SMA, vimentin, and desmin in liver tissue. Specifically, mouse liver tissue was fixed with 4% polyformaldehyde, embedded in paraffin, and sectioned to a thickness of 4 μ m. The sections were then deparaffinized in xylene, rehydrated through a graded ethanol series, and subjected to antigen retrieval. Afterwards, the sections were blocked with 5% bovine serum albumin to reduce nonspecific binding. After blocking and washing with phosphate buffered saline, each section was incubated with the primary antibody (against α -SMA, vimentin, or desmin) in a humid chamber at 4°C overnight. The sections were then washed three times with phosphate buffered saline, incubated with an horseradish peroxidase-conjugated secondary antibody at room temperature for 30 min, mounted with a mounting medium, and observed under a microscope.

2.7. Western blot analysis

Liver tissue samples were homogenized in RIPA lysis buffer and then the protein concentration was determined using a BCA assay kit from Cwbio. Each protein sample (10 μ g) was separated by SDS-PAGE and transferred to a PVDF membrane, which

was then blocked with 5% skim milk at room temperature for 2 h. The membrane was subsequently incubated overnight at 4°C with specific antibodies: α -SMA (1:1 000), vimentin (1:1 000), p-PI3K (1:1 000), PI3K (1:1 000), p-AKT (1:1 000), AKT (1:1 000), p-GSK3 β (1:1 000), GSK3 β (1:1 000), and GAPDH (1:5 000). On a subsequent day, the membranes were washed thrice with PBST and then incubated with HRP-labeled goat anti-rabbit IgG (1:5 000) for 1 h at room temperature. Following PBST washing, the protein bands were visualized with an enhanced chemiluminescence reagent and captured using a Bio-Rad imaging system. Band intensities were subsequently quantified using Image-Pro Plus 6 software.

2.8. Statistical analysis

All data are presented as mean $\pm$ standard error of the mean (SEM) and were statistically assessed using one-way analysis of variance followed by LSD multiple comparisons tests using SPSS 22.0 (SPSS, Armonk, New York, USA), with the significance threshold set at a *P*-value below 0.05.

2.9. Ethical statement

All experimental procedures were performed according to the National Institutes of Health (NIH) guide for the care and use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and were approved by the Animal Care Committee of Henan University of Traditional Chinese Medicine (IACUC-S202307002, dated 12 July 2023).

3. Results

3.1. Serum analysis

As shown in Figure 1, the serum levels of ALT (*P* < 0.001), AST (*P* < 0.001), ALP (*P* < 0.05), and TBil (*P* < 0.05) were significantly elevated in the model group following CCl₄ administration compared to the normal control group. However, treatment with apigenin markedly reduced these enzyme levels and TBil content (*P* < 0.05).

3.2. Histopathological results

The H&E staining results revealed distinct differences between the normal control and model groups (Figure 2). In the normal control group, hepatocytes exhibited a regular morphology, hepatic lobules displayed clear and intact structures, and liver tissue cells were arranged radially around the central vein, with no noticeable fibrous connective tissue proliferation. Conversely, the model group exhibited swollen hepatocytes, some displaying vacuolar changes

or fatty degeneration, and disorganized arrangements. Hepatocyte necrosis and inflammatory cell infiltration were observed in localized areas. Comparatively, the apigenin-treated groups showed milder vacuolar changes and significant reductions in hepatocytes with fatty degeneration. Moreover, varying degrees of reduction in inflammatory cell infiltration were noted. Notably, the degree of fibrous proliferation was significantly attenuated, particularly in the high-dose apigenin group, where hepatocytes exhibited near-normal morphology.

Using the Scheuer staging system, it was found that the grades of the model group were generally above grade 3, indicating extensive fibrosis. After the administration of apigenin, the grades were reduced to below grade 2, showing a significant improvement in the degree of fibrosis (Figure 2B).

3.3. Apigenin reduces CCl₄-induced collagen expression in mouse liver

As shown in Figure 3A and B, the normal control group had

minimal collagen fibers around the central vein and portal area. In contrast, the model group exhibited a substantial amount of red collagen fibers at the periphery of the lobules, around the portal area, and the central vein, indicating the presence of liver fibrosis. However, in the treatment groups, the collagen fibers around these areas significantly decreased. Quantitative analysis using Image-Pro Plus software revealed that the Sirius Red-positive area in the model group was significantly higher than in the normal control group ($P < 0.001$). Additionally, treatment with apigenin dose-dependently induced a marked reduction in the Sirius Red-positive areas compared with the model group ($P < 0.001$).

A significant increase in hydroxyproline content was found in the liver of the model group ($P < 0.05$), while all doses of apigenin significantly reduced hydroxyproline levels in the fibrotic liver ($P < 0.05$) (Figure 3C).

Immunohistochemical results demonstrated that compared to the normal control group, the expression of α -SMA ($P < 0.001$), vimentin ($P < 0.001$), and desmin ($P < 0.05$) around the portal area and between hepatic plates was significantly elevated in the model

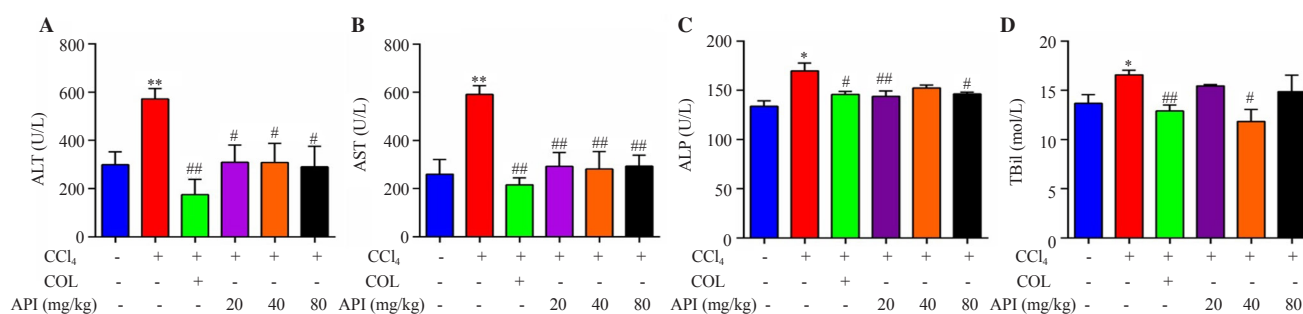


Figure 1. Effect of apigenin on serum ALT, AST, ALP, and TBil in mice with CCl₄-induced liver fibrosis. The data are represented as mean $\pm$ SEM ($n=6$). * $P < 0.05$, ** $P < 0.001$ vs. the normal control group; # $P < 0.05$, ### $P < 0.001$ vs. the CCl₄ group. One-way ANOVA followed by LSD *post hoc* test was performed for statistical analysis. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; TBil: total bilirubin; COL: colchicine; API: apigenin.

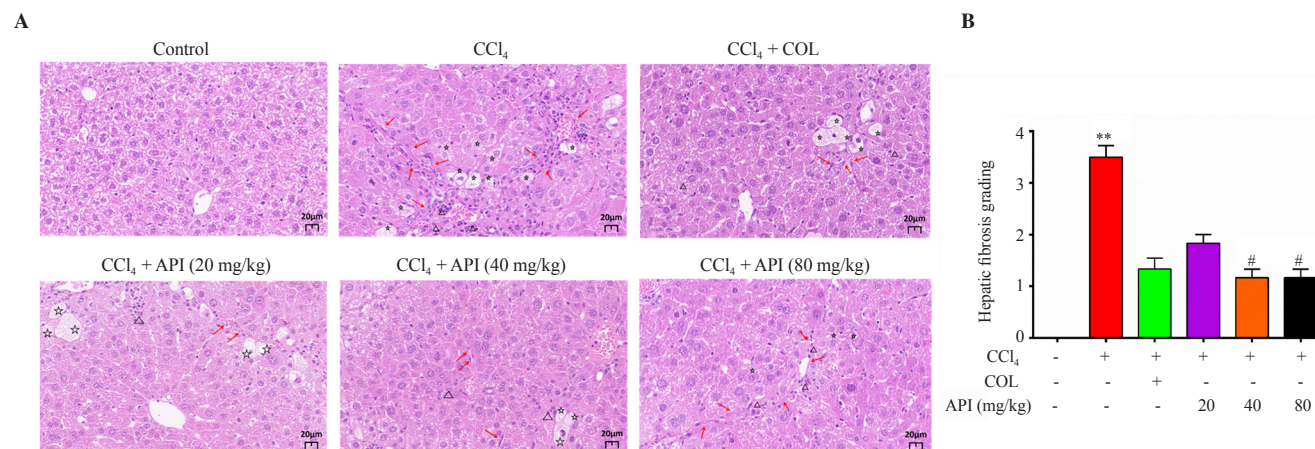


Figure 2. Apigenin ameliorates liver damage in mice with CCl₄-induced liver fibrosis. (A) H&E staining (magnification: 40 $\times$; scale bar: 20 μ m). (B) The quantitative analysis of H&E staining. Red arrow: hyperplastic fibrous connective tissue; triangle: inflammatory cell infiltration; pentagram: cell swelling, foamy degeneration. The data are represented as mean $\pm$ SEM ($n=3$). ** $P < 0.001$ vs. the normal control group; # $P < 0.05$ vs. the CCl₄ group. One-way ANOVA followed by LSD *post hoc* test was performed for statistical analysis.

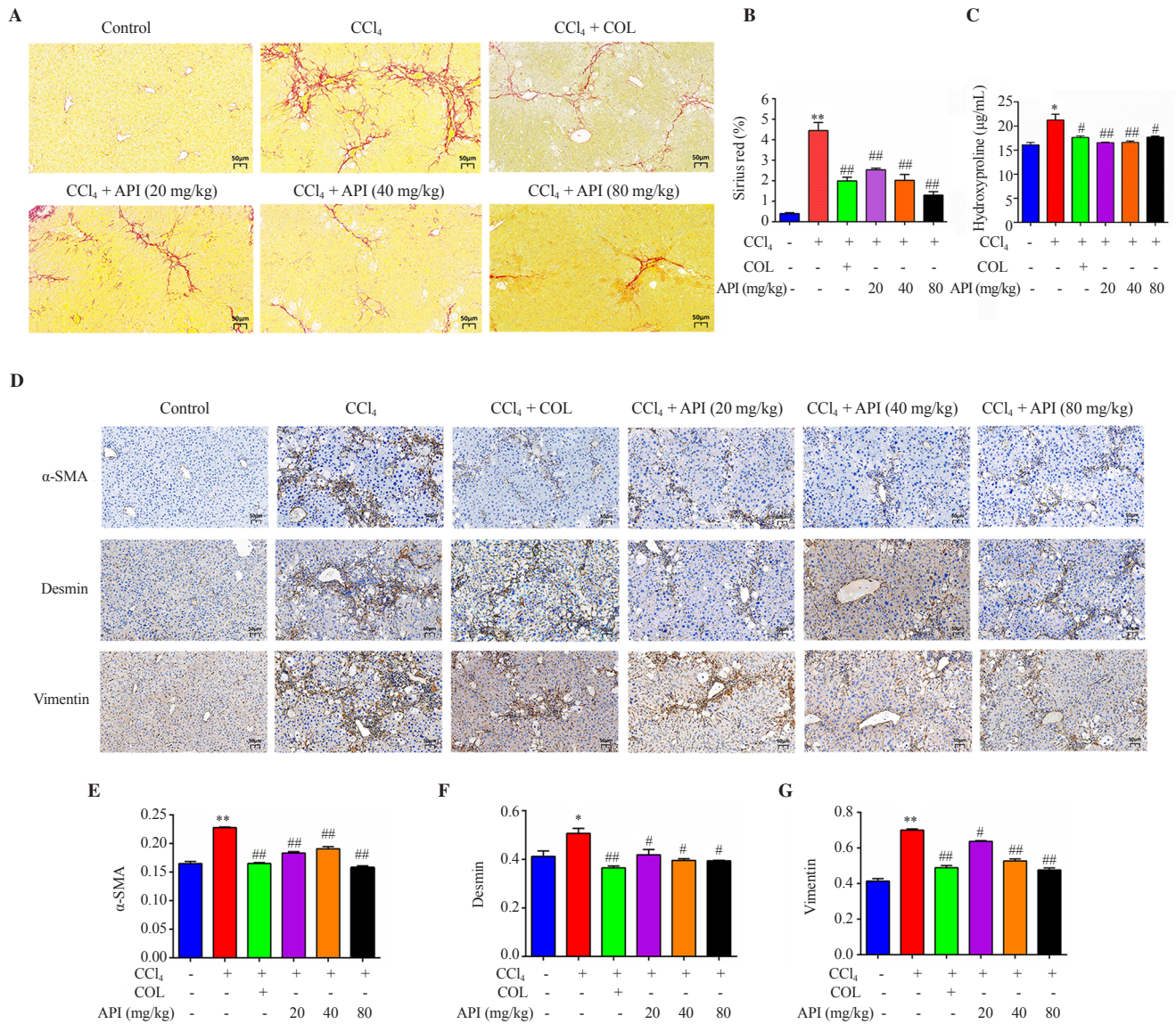


Figure 3. Apigenin reduces CCl₄-induced collagen expression in mouse liver. (A) Sirius Red staining (magnification: 20 ×; scale bar: 50 μm). (B) The quantitative analysis of Sirius Red staining. (C) The content of hydroxyproline. (D) The immunohistochemical staining of α-SMA, vimentin, and desmin in the liver (magnification: 20 ×; scale bar: 50 μm). (E-G) The quantitative analysis of immunohistochemical staining of α-SMA, vimentin, and desmin. The data are represented as mean ± SEM (*n*=3). **P* < 0.05, ***P* < 0.001 *vs.* the normal control group; #*P* < 0.05, ##*P* < 0.001 *vs.* the CCl₄ group. One-way ANOVA followed by LSD *post hoc* test was performed for statistical analysis.

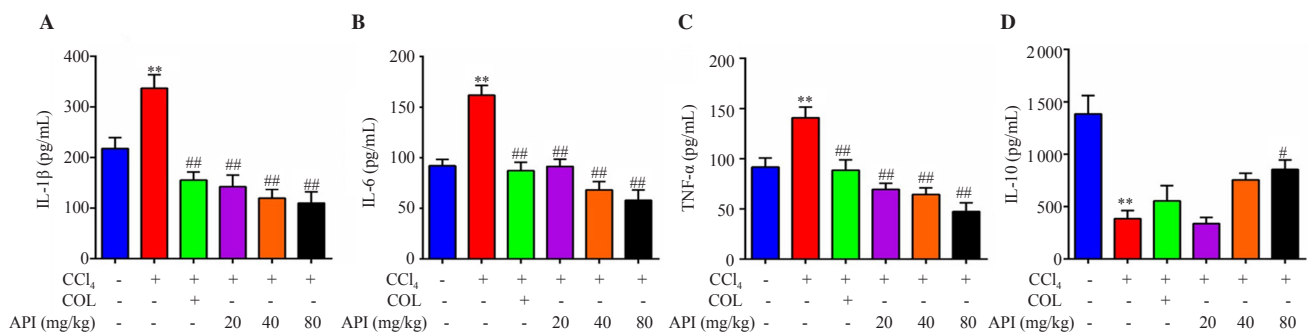


Figure 4. Apigenin inhibits CCl₄-induced hepatic inflammation. The levels of (A) IL-1β, (B) IL-6, (C) TNF-α, and (D) IL-10 were measured by ELISA. The data are represented as mean ± SEM (*n*=6). ***P* < 0.001 *vs.* the normal control group; #*P* < 0.05, ##*P* < 0.001 *vs.* the CCl₄ group. One-way ANOVA followed by LSD *post hoc* test was performed for statistical analysis.

group. However, apigenin treatment lowered the expression of α -SMA ($P < 0.001$), desmin ($P < 0.05$), and vimentin ($P < 0.05$ for the low dose group and $P < 0.001$ for the medium and high dose groups). This suggests that collagen fiber deposition occurred in the liver of the CCl_4 group, which could be alleviated by apigenin (Figure 3D-G).

3.4. Effect of apigenin on CCl_4 -induced hepatic inflammation

As seen in Figure 4, a notable elevation in pro-inflammatory cytokines, including IL-1 β ($P < 0.001$), IL-6 ($P < 0.001$), and TNF- α ($P < 0.001$), and a marked reduction in IL-10 ($P < 0.001$) level were induced by CCl_4 administration. In contrast, apigenin showed a dose-dependent decrease in these markers and an increase in IL-10 level.

3.5. Effect of apigenin on CCl_4 -induced hepatic oxidative stress

Figure 5 illustrates the effect of apigenin on CCl_4 -induced hepatic oxidative stress. In the CCl_4 -administered group, there was a significant decrease in the activities of SOD ($P < 0.001$) and catalase ($P < 0.001$), and total antioxidant capacity ($P < 0.001$), while the levels of MDA ($P < 0.001$) were increased. However, apigenin reduced CCl_4 -triggered oxidative stress by decreasing MDA levels and increasing SOD and catalase activities.

3.6. Apigenin relieves CCl_4 -induced hepatic fibrosis via downregulating PI3K/AKT/GSK3 β pathways

As shown in Figure 6, consistent with the immunohistochemistry

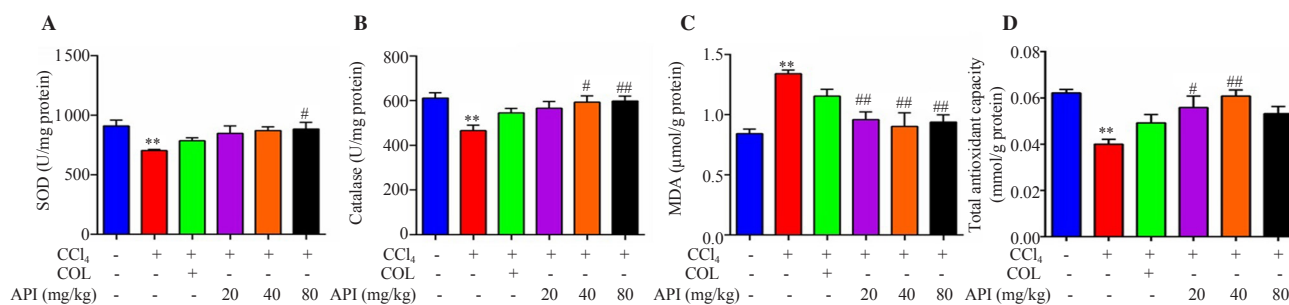


Figure 5. Apigenin reduces CCl_4 -induced hepatic oxidative stress. The activities of (A) SOD and (B) catalase, (C) MDA level as well as (D) total antioxidant capacity were measured by corresponding assay kits. SOD: superoxide dismutase; MDA: malondialdehyde. The data are represented as mean $\pm$ SEM ($n=6$). ** $P < 0.001$ vs. the normal control group; # $P < 0.05$, ## $P < 0.001$ vs. the CCl_4 group. One-way ANOVA followed by LSD *post hoc* test was performed for statistical analysis.

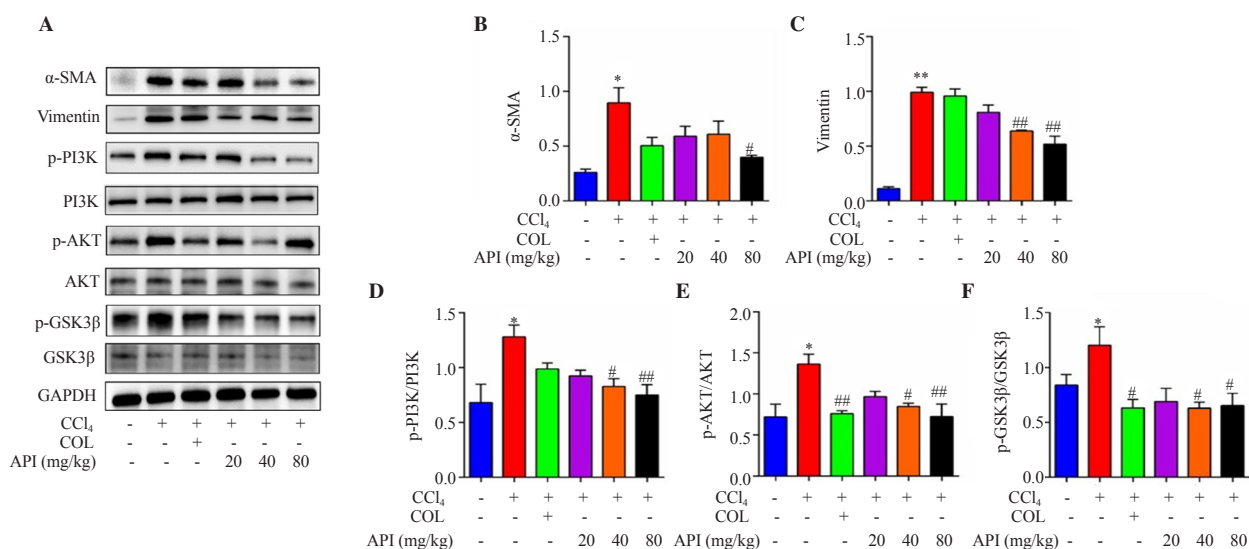


Figure 6. Apigenin relieves CCl_4 -induced hepatic fibrosis via PI3K/AKT/GSK3 β pathways. (A) Representative blots. (B-F) The protein expression levels of α -SMA, vimentin, p-PI3K/PI3K, p-AKT/AKT, and p-GSK3 β /GSK3 β were determined by Western blot. The data are represented as mean $\pm$ SEM ($n=6$). * $P < 0.05$, ** $P < 0.001$ vs. the normal control group; # $P < 0.05$, ## $P < 0.001$ vs. the CCl_4 group. One-way ANOVA followed by LSD *post hoc* test was performed for statistical analysis.

results, the Western blot analysis revealed that CCl₄ administration increased the protein expression of α -SMA and vimentin in the liver. In contrast, apigenin treatment significantly reduced this increase. Furthermore, CCl₄ administration significantly upregulated the ratios of p-PI3K/PI3K, p-AKT/AKT, and p-GSK3 β /GSK3 β in the liver. Apigenin significantly inhibited these upregulations, suggesting that its anti-liver fibrosis effect may be related to the inhibition of the PI3K/AKT/GSK3 β signaling pathway.

4. Discussion

Characterized by multiple pathological mechanisms, liver fibrosis develops through sustained damage to hepatocytes, compromised epithelial/endothelial barriers, and the subsequent release of pro-inflammatory signaling molecules. This cascade activates collagen-producing myofibroblasts in the liver, driving abnormal deposition of type I collagen and other ECM components that ultimately form rigid scar tissue[14]. The regression of liver fibrosis is the main goal of clinical treatment, as it promotes recovery of liver function and reduction of portal pressure, thus reducing the incidence of portal hypertension complications[15]. The attenuation of fibrosis manifests through coordinated biological processes: downregulation of cytokine networks that drive inflammation and matrix production; enzymatic clearance of accumulated collagens through upregulated proteolytic pathways; apoptosis of activated myofibroblast populations; and progressive remodeling of established fibrotic lesions *via* MMP activity.

The present study delineates apigenin's multifaceted therapeutic potential in attenuating CCl₄-induced hepatic fibrosis through integrated molecular, histopathological, and biochemical analyses. The observed reduction in serum hepatotoxicity markers and restoration of liver architecture in the apigenin-treated groups underscore its hepatoprotective capacity. Notably, the dose-dependent attenuation of collagen deposition, as quantified *via* Sirius Red-positive areas and hydroxyproline content, directly implicates the beneficial role of apigenin in suppressing ECM remodeling, a hallmark of fibrotic progression.

The transdifferentiation of HSCs into myofibroblasts is a key event in the fibrotic remodeling of the liver. Exposure to profibrotic signaling molecules triggers this cellular transformation, characterized by significant cytoskeletal reorganization, as evidenced by increased expression of α -SMA and overexpression of vimentin. Activated HSCs migrate to the sites of hepatic injury and secrete ECM, ultimately leading to the formation of permanent scar tissue[16,17]. Our findings suggest that the anti-fibrotic efficacy of apigenin is mechanistically linked to its inhibition of HSC activation, as evidenced by diminished expression of α -SMA, vimentin, and

desmin.

Inflammation is a major feature of chronic liver disease and plays a key role at any stage of the fibrotic process, even during fibrotic remission. The progression of liver fibrosis is critically driven by pro-inflammatory mediators (IL-6, TNF, and TGF β) with dual profibrogenic and inflammatory functions. Pathologically, oxidative stress serves as a central mechanistic hub in fibrogenesis, wherein sustained exposure to injurious stimuli induces pathological reactive oxygen species accumulation, culminating in cellular dysfunction through structural and metabolic perturbations[18]. Experimental evidence confirms that targeted attenuation of oxidative insults not only arrests but partially reverses established fibrosis in preclinical models[19,20]. Cellular redox homeostasis is maintained by endogenous defense systems, including SOD, catalase, and glutathione, which play a crucial role in protecting against hepatic inflammation through reactive oxygen species neutralization[21]. These redox regulators effectively neutralize superoxide radicals and hydrogen peroxide, thereby reducing oxidative damage. In our experimental model, apigenin attenuated CCl₄-induced increases in pro-inflammatory mediators (IL-1 β , IL-6, and TNF- α) while enhancing SOD and catalase activities. This was associated with a reduction in MDA levels and an increase in total antioxidant capacity, highlighting apigenin's dual antioxidant and anti-inflammatory effects, which enhance its antifibrotic efficacy.

Mechanistically, PI3K triggers signaling cascades by converting PIP2 to PIP3, which recruits Akt to the membrane. Phosphorylation by PDK activates Akt[22,23]. Activated Akt transmits signals through downstream effectors such as GSK3 β , regulating critical cellular processes from proliferation to apoptosis[24]. Targeting this pathway therapeutically inhibits TGF- β 1-driven ECM overproduction and myofibroblast activation[25], with TRPC6 knockout models showing similar antifibrotic effects through modulation of the PI3K/AKT/GSK3 β axis[26]. Akt-mediated serine-9 phosphorylation regulates GSK3 β activity, a mechanism crucial for fibrogenesis[27]. Importantly, inhibiting GSK3 β has emerged as a promising antifibrotic strategy, as it suppresses β -catenin signaling and impedes HSC activation and proliferation[28]. GSK3 β plays a multifaceted role in the pathological pathways of liver fibrosis, impacting early inflammatory injury, cell activation, the epithelial-mesenchymal transition, and ECM deposition[29–31]. Enhancing GSK3 β activity can suppress β -catenin expression, restraining HSC activation and proliferation, which helps prevent fibrogenesis[28]. Modulating the Akt/GSK3 β /Nrf2 signaling cascade has shown promise *in vitro*, inhibiting EMT and HSC activation in cellular and mouse models of liver fibrosis[32]. Therefore, modifying the PI3K/AKT/GSK3 β pathway appears to be a viable strategy for mitigating HSC activation and subsequent fibrosis. Our research findings indicate that apigenin inhibits the PI3K/AKT/GSK3 β signaling pathway,

thereby not only interrupting HSC activation but also impeding the downstream transcriptional programs governing ECM production.

While these findings position apigenin as a promising candidate for antifibrotic therapy, several considerations warrant further investigation. First, the pharmacokinetic profile of apigenin, including its bioavailability and tissue distribution, needs optimization to enhance therapeutic efficacy. Second, the crosstalk between the PI3K/AKT/GSK3 β pathway and other pro-fibrogenic pathways (*e.g.*, TGF- β and Wnt/ β -catenin) should be investigated to elucidate apigenin's broader mechanistic network. Finally, preclinical validation in advanced fibrosis models and human-derived HSCs is necessary to strengthen translational relevance.

In conclusion, this study provides compelling evidence that apigenin ameliorates hepatic fibrosis through multiple mechanisms, primarily by inhibiting the PI3K/AKT/GSK3 β pathway. These insights advance our understanding of flavonoid-based antifibrotic therapies and highlight apigenin's potential as a targeted intervention for liver fibrosis.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

TS designed the study and supervised the project. BW collected the data and drafted the manuscript. XL and XZ conducted data analysis and interpretation. YZ performed the analytical calculations. SY and NL contributed to the final approval of the version to be published.

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