

Dencichine ameliorates hepatic fibrosis by modulating the PI3K/AKT signaling pathway in cellular and murine models

Longkuan Li¹, Lixian Mo², Huan Luo², Zeyun Liu², Wei Zhang^{2*}, Jinyou Tian^{3*}, Fuxue Meng^{2*} and Yingqiang Chen^{3*}

¹Department of Pharmacy, The Third Affiliated Hospital of Guizhou Medical University

²Medical Experimental Center, The Third Affiliated Hospital of Guizhou Medical University

³Department of Infectious Diseases, The Third Affiliated Hospital of Guizhou Medical University

Abstract: Background: Fibrotic liver is one of the serious pathophysiological consequences of chronic injury to the liver, may progress toward serious complications including cirrhosis and hepatic carcinoma. **Objectives:** *Panax notoginseng* is used for both therapeutic and prophylactic purposes in TCM; it is a source of Dencichine, an active substance that has been proven to exhibit antifibrotic properties in hepatic fibrosis. The present study investigated how Dencichine treatment ameliorates liver fibrosis using an animal model and a cellular fibrosis model. **Methods:** Randomly assigned, male C57BL/6J mice received 10% CCl₄ for 15 consecutive weeks to induce hepatic fibrosis. Treatment with Dencichine was initiated from 7th week onward. To simulate *in-vitro* the presence of a fibrotic microenvironment, LX-2 cells were treated with PDGF-BB at a concentration of 20 ng/mL and incubated from one up to four days and then treated by various doses of Dencichine. Liver function was evaluated by measuring alanine aminotransferase (ALT), AST, HA and LN. Histopathology was evaluated by haematoxylin/eosin (HE), Masson's trichrome and Sirius red stains. Quantitative real-time PCR and western blot analyses were used to evaluate mRNA and protein expression levels, respectively. Wound-healing assays and transwell chamber experiments were used to assess cell migration; EdU proliferation detection and flow cytometry were performed to measure cell proliferation and cell cycle, respectively. **Results:** The most important results are a considerable reduction of serum concentrations of AST and ALT, HA and laminin in CCl₄-treated mice decreased liver injury and ECM deposition. Furthermore, this compound downregulated the expression of cyclin D1 and cyclin E1 and blocked the activation of PI3K and AKT kinases in fibrotic liver cells. **Conclusion:** These results indicate that Dencichine may slow the development of liver fibrosis by interfering with the PI3K-AKT signaling cascade, demonstrating significant anti-scarring properties.

Keywords: Dencichine; Liver fibrosis; Phosphatidylinositol 3-kinase-AKT serine/threonine kinase pathway signaling

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INTRODUCTION

Liver cirrhosis is the progressive result of chronic liver disease and is characterized with excessive deposition of ECM in liver tissue, which results in dysfunction and destruction of the organ and is responsible for the development of advanced hepatic disease, being one of the main causes of mortality worldwide (Xi *et al.*, 2024). According to global epidemiological statistics, about 800 million people suffer from liver diseases every year and more than 2 million patients die every year (Marcellin & Kutala *et al.*, 2018). Although great progress has been made in research on the mechanisms of liver fibrosis and many potential drugs for liver fibrosis have been identified, no approved drug specifically targeting fibrosis is currently available. Existing therapies focus mostly on reversing the cause of the fibrotic damage i.e., viral suppression or abstinence from drinking in case of alcoholic liver disease. (Tao *et al.*, 2025). Existing anti-fibrotic drugs suffer from multiple limitations compared with standard drug treatment typically.

Currently available therapies target single molecules and are thus not well suited to modulate the complex pathophysiology underlying progressive fibrosis. Moreover, they may present adverse events severely limiting their application in the clinic. To overcome the shortcomings of current Western medicines, novel therapeutic strategies are urgently needed. With thousands of years of clinical practice, TCM offers a vast reservoir of bioactive natural products. These compounds have shown unique advantages in modulating complex diseases and thus represent a promising pharmacological avenue. In recent years, it has been found that many traditional Chinese medicines and their preparations have certain advantages in the prevention and treatment of liver fibrosis and have achieved certain clinical effects. For example, tanshinone compounds in *Salvia miltiorrhiza* can inhibit the activation and proliferation of hepatic stellate cells (HSCs), reduce collagen synthesis and thus alleviate liver fibrosis (Li *et al.*, 2024 ; Fu *et al.*, 2024 , Shi *et al.*, 2026) . Huperzine A can exert anti-liver fibrosis effects by regulating oxidative stress and inflammatory

*Corresponding authors: e-mail: 1042410740@qq.com; mfx5675143@126.com; 1804941600@qq.com; 482393942@qq.com

responses (Luo *et al.*, 2024). The above studies indicate that traditional Chinese medicine offers new approaches for the prevention and treatment of liver fibrosis. Panax notoginseng is one of the traditional precious Chinese medicinal materials, with functions including promoting blood circulation, removing blood stasis, stopping bleeding, relieving pain and reducing swelling (Xu *et al.*, 2023). More and more studies have shown that it has a good therapeutic effect on liver fibrosis.

It was reported that Panax notoginseng, the major active component Dencichine, could treat liver fibrosis (Peng *et al.*, 2009). This herb significantly reduced serum fibrosis markers in animal experiments, prevented inflammatory responses and inhibited the activation and proliferation of HSCs, thereby alleviating liver fibrosis (Gong *et al.*, 2022). Notably, the pathogenesis of many chronic or complex diseases involves dysregulation of several key signaling pathways, including PI3K/AKT, TGF- β /Smad and MAPK. Active compounds derived from TCM have been shown to exert pharmacological effects, including anti-inflammation, anti-fibrosis, apoptosis induction and proliferation inhibition, by modulating these pathways. However, they are poorly understood. In this study, we use parallel experimental models (*in-vivo* and *in-vitro*) to investigate the effect of Dencichine on the pathogenesis of liver fibrosis. Our findings should provide a basis for the application of Dencichine as an anti-fibrosis therapy and identify potential therapeutic target molecules for new drugs against fibrosis.

MATERIALS AND METHODS

Main reagents

The following reagents were used for the current study: Dencichine (purity $\geq 98\%$, CAS: 5302-45-4, Solarbio, Beijing, China); CCl₄ (Nanjing Reagent, C0731530924, Nanjing, China) which is diluted at 10% concentration into olive oil as experiment modeling; ALT (Nanjing Jiancheng, C009-2-1, Nanjing, China), AST (Nanjing Jiancheng, C010-2-1, Nanjing, China), HA (Solarbio, Beijing, China), LN (Solarbio, Beijing, China); Histological Staining Kits HE, Masson and Sirius Red (MCE Life Sciences, USA); a-SMA, TGF- β 1, Smad2/3, p-Smad2/3, PI3K, AKT, collagen 1 and TIMP1 antibodies (MCE Life Sciences, USA); Horseradish peroxidase(HRP) labeled goat anti-rabbit IgG second antibody (Solarbio, Beijing, China); RIPA cell lysis solution, BCA protein assay kit, SDS-PAGE gel preparation system and ECL luminescence detection kit (Beyotime, Shanghai, China); Trizol extraction reagent (Solarbio, Beijing, China), cDNA synthesis kit and SYBR Green real-time PCR detection system (Thermo Scientific, USA).

Methods

In-vivo mouse model of liver fibrosis and *in-vitro* human liver cell culture models

Forty C57BL/6 J mice (male) aged 6-7 weeks and weighing 16-20 g were purchased from Nanjing Junke Biotechnology Co., Ltd., Nanjing, China). After delivery, the mice were allowed to acclimatize for 7 days under specific pathogen-free (SPF) conditions, at room temperature (22 $\pm$ 2°C) and relative humidity (50 $\pm$ 10%). Mice were stratified by body weight into light-, medium- and heavy-weight strata. Animals in each stratum were numbered and randomly allocated to experimental groups via lottery-style drawing within each stratum, they were divided at random in groups of ten mice for four different experiments (untreated), a disease model group and two treatment groups with different concentrations of dencichine. For the first three months, except for controls, they received an i.p. injection of either pure olive oil or 10% CCl₄ dissolved in olive oil every other week, with a dose of 5 μ L/g BW. From day 50 onwards (end of week 7), the treatment groups received Dencichine dissolved in 0.9% NaCl, orally, for 8 consecutive weeks, with a fixed dose volume of 0.2 mL per 20 g of body mass. The low-dose group received 2.925 mg/kg/day of dencichine and the high-dose group received significantly more of this compound.

The rats were treated with the extract at a dosage level of 11.7 mg/kg/day, orally once daily for 8 weeks (Gong *et al.*, 2022) and then the animals were euthanized. The animals were anesthetized with 0.3 percent sodium pentobarbital by ip injection (0.05g/kg) before sampling and blood and liver tissue samples were collected properly. The study was approved by the Ethics Committee of The Third Affiliated Hospital of Guizhou Medical University (No. 2022A03). For cell experiments, the Hepatic stellate cell line LX-2 was treated with 20 ng/mL PDGF-BB (platelet-derived growth factor). With an incubation period of 1-48 h, according to the parameter analyzed (Yao *et al.*, 2024) Group 1 was administered dencichine (0.01 mM), Group 2 was administered dencichine (0.1 mM) and Group 3 was administered dencichine (1 mM).

Biological sample acquisition

At the end of the treatment (day 56), animals fasted overnight and received an intraperitoneal injection of the aforementioned sodium pentobarbital for anaesthesia and then sacrificed by orbital sinus puncture method. The suspensions were placed in sterile centrifuge tubes and left undisturbed at room temperature for 2 hours before being centrifuged (4°C, 3000 r.p.m.) for 10 min to collect the serum fraction. The collected serum samples were then divided into small portions and stored in a freezer (-80°C) until used for biochemical analysis. Animals that reached the experimental endpoint or could not continue the experiment were immediately euthanized under anesthesia by injection of sodium pentobarbital.

All the mice were quickly sacrificed and their livers were isolated. The livers were washed with chilled normal saline and dried on filter paper. A small piece of the left liver was placed in a 10% neutral formaldehyde solution for pathological analysis (HE staining, Masson staining and violet blue staining) to assess the severity of hepatitis and fibrosis. The other part of the liver tissue was wrapped in aluminum foil and immediately frozen in liquid nitrogen at high speed, then stored at -80°C for future use. The expression of corresponding proteins and genes was analyzed by Western Blot and real-time fluorescence quantitative PCR (qRT-PCR).

Serum liver function and liver fibrosis index testing

ALT and AST activity levels in blood serum were measured with a fully automated biochemical analysis system (BIOBASE, Shandong, China). To test several serum indicators, such as HA, LN, TGF- β 1, IL-6, TNF- α , INF- γ , MDA and PDGF-BB, the ELISA method has been applied.

Histopathological examination of liver tissue

Liver tissues were fixed, embedded in paraffin wax and sectioned. Sections were stained with H&E to evaluate the liver morphology under a microscope. Light microscopy was used to assess cellular morphology and determine the degree of hepatocyte injury, cell necrosis and inflammation. Masson's trichrome was used for the analysis of collagen fibers and image processing for colorimetric determination of staining intensity was performed in the ImageJ program to evaluate fibrosis levels. Sections were analyzed by polarizing microscopy after Sirius Red staining, where the red birefringence and green color were from type I collagen and III collagen, respectively. This allowed us to observe variation in number and localization among the different types of collagen, which can provide some insight into the pattern of fibrosis.

Western blot analysis of protein expression

To isolate the proteins, about 20 mg of liver tissues were fully homogenized and lysed. Similarly, Hepatic stellate cells were isolated by centrifugation and lysed for protein isolation. Protein separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by electroblotting to polyvinylidene fluoride membranes (Millipore Ltd.) on an electrophoresis system from Anneng Biotechnology. After being blocked, the membranes were incubated overnight at 4°C with appropriate primary antibody diluted as follows: α -SMA (1:1000). Type I collagen, Smad2/3, phosphorylated Smad2/3, platelet-derived growth factor receptor β , matrix metalloproteinase 2, matrix metalloproteinase 9, tissue inhibitor of metalloproteinases 1 and glyceraldehyde-3-phosphate dehydrogenase (1:1000). Horseradish peroxidase-conjugated secondary antibody (anti-rabbit mouse IgG, 1:5000) were added and incubated at room temperature for 1 hour; the loading control is GAPDH

(1:5000). The normalized value of the sample group was calculated.

RT-PCR for gene expression analysis

Total RNA from HSCs and liver tissues were extracted using the TRIzol-chloroform method. According to instructions of Bestar qPCR RT Kit, reverse transcriptase reactions were set up by the investigators; after the RT reaction had been completed and before the cDNA was stored at -20°C, qPCR reactions (20 μ L) were prepared according to manufacturer instructions. Gene expressions are quantified with a fluorescence-based Agilent Stratagene Mx3000P Real-Time PCR System. Data are analyzed using relative quantitative $2^{-\Delta\Delta Ct}$ method. The primer sequences used for each specific gene are listed below.

Cell proliferation assay

The human hepatic stellate cell line LX-2 was obtained from Kunming Cell Bank (Kunming, China). Cells were cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin and 100 μ g/mL streptomycin, at 37°C in a humidified atmosphere containing 5% CO₂. All cells were used at passages 4-5 after thawing for the experiments. Cells were routinely passaged upon reaching 80-90% confluence using 0.25% trypsin-EDTA. Cells were resuspended in PBS at a density of 1×10^6 cells/mL and we finally cultured them in a 96-well plate (in each well, 1.5×10^3 cells were seeded into a 100 μ L volume of cell culture medium). Three technical replicates were performed per treatment group. Different concentrations of dencichine (0.01 mM, 0.1 mM and 1 mM) were used on the cells for various time periods (24, 48 and 72 h). Proliferation of cells were analyzed by cell counting kit (CCK)-8 after following instructions in the manual from the supplier and absorbance was measured at 450 nm. For the EdU incorporation assay, exponential-phase cell cultures were incubated with 30 μ M EdU for 12 h. After that the cell culture was collected, fixed in 4% paraformaldehyde (room temperature for 15 min) followed by two washes in PBS. For membrane permeabilization, 0.5% Triton X-100 for 15 min, then clicked with Click reaction solution for 30 min at room temperature in the dark. After two additional PBS washes, the samples were analyzed by flow cytometry. Before flow cytometry, cells were pelleted by centrifugation and resuspended in PBS (at a concentration of 1×10^6 cells/mL). The cells were suspended individually, kept in suspension and a fluorescent antibody was added. The cells are then incubated with the fluorescent antibody (for 30 minutes) at room temperature in the dark. After incubation, two PBS washes to remove unbound antibodies; the cells were then resuspended in FC buffer, filtered and analyzed by flow cytometer.

Table 1: The primer sequences.

ID	Sequence F (5'-3')	Sequence R (3'-5')
GAPDH	CCTCGTCTCATAGACAAGATGGT	GGGTAGAGTCATACTGGAACATG
CyclinE1	AGTACCCACAGCAGGTCTTC	CTGCATCAACTCCAACGAGG
CyclinD1	AAGTGTGACCCGGACTGC	AGCTTCTTCCTCCACTTCCC
Timp1	CTCTGGCATCCTCTTGTTC	GCCCTTATAACCAGGTCCGA
Mmp9	TCGGATGGTTATCGCTGGTG	AAGACGCACATCTCTCCTGC
Mmp2	GAACACCATCGAGACCATGC	CATGCTCCCATCGACCAAAG
α -SMA	GGGCCAAAAGGACAGCTATG	TGATGCCGTGTTCTATCGGA
PDGF- β R	TCGAGCCAAGACACCTCAAA	AGTGCCTTCTTGTCATGGGT
CollagenI	GAGAGAGCATGACCGATGGA	GTAGGCTACGCTGTTCTTGC

Cell migration assay

Wound healing assay Cell suspensions were seeded into 6-well plates with a density of 5×10^5 cells/well and incubated for 24 h (or longer if needed) until they reached confluence. An artificial scratch was made through the confluent cell monolayer using a sterile 200 μ l pipette tip pulled down along the bottom edge of the plate. After washing away floating debris with PBS, photographs of the same wound area were taken before, after 12 h of treatment and after 2 days of treatment. Wound closure was quantified using ImageJ analysis software.

In the Transwell chamber for cell migration evaluation, cells were collected using serum-free culture medium. About 50,000 cells were seeded in the top chamber and allowed to migrate at 24 or 48 h, as described above. The migrated cells on the bottom-side membranes were fixed with 4% paraformaldehyde (PFA) for 30 minutes, followed by 20-min staining in 0.1% crystal violet solution. The number of cells that passed through the pores (translocated) was counted under a light microscope at $\times 5$ magnification for each cell culture in five random microscopic fields.

Data analysis

All experiments were performed with 3 biological replicates per group. Normality of the data was assessed using the Shapiro-Wilk test and the data were found to be normally distributed ($p > 0.05$ for all groups). A one-way ANOVA was therefore used for multiple comparisons. All statistical analyses were performed using SPSS 22 and GraphPad Prism 8.0 and the significance threshold was set at $p < 0.05$.

RESULTS

Dencichine Prevents CCl₄-Induced Liver Fibrosis in Mice
To study the effect of dencichine on hepatic fibrosis, we chose to use an animal model (mouse C57BL/6J) and analyzed biochemical markers including ALT, AST, HA and LN ($P < 0.01$) in the experimental groups compared with normal control indicating that CCl₄ induced severe liver damage; treatment with dencichine significantly abolished the additive inhibitory effect the elevated levels

of these parameters induced by CCl₄ (Fig. 1A). Observed microscopically with H&E, Masson's trichome and Sirius red stained for wide tissue injury, inflammation response and collagen accumulation of the CCl₄ treated groups (Fig. 1B). Western blotting and real-time polymerase chain reaction (RT-PCR) analysis showed that the expression level of alpha-smooth muscle actin (α -SMA), which is a marker for activated HSCs was significantly increased. In our experiment, we found that, compared with the control group, Collagen I was significantly increased in the model group and PDGFR- β was also increased. MMP2, MMP9 and TIMP1. It is also interesting that treatment with dencichine significantly attenuated the levels of all three molecules: as shown in figures. 1C, D.

The ELISA results further confirmed that levels of TGF- β 1, IFN- γ , TNF- α , MAD and PDGF-BB. On the other hand, the use of dencichine was able to control the secretion of these factors (Fig. 2).

Dencichine inhibits the activation of HSCs induced by PDGF-BB background

To examine whether dencichine affects liver function, hepatic stellate cells were activated by PDGF-BB prior to treatment with different concentrations of dencichine. The results showed that dencichine inhibited hepatic stellate cell proliferation in a concentration-dependent manner, markedly inhibited cell migration and induced cell cycle arrest (Figs. 3A-3F).

Dencichine reduces hepatic fibrosis through the PI3K/AKT signaling pathway in an in-vitro model

Given that the PI3K/AKT pathway is involved in HSC survival and proliferation, we hypothesized that it might be one of the most important pathways targeted by dencichine and further investigated its underlying mechanism of anti-fibrosis in a cell model *in vitro*. Immunoblots showed that treatment with increasing concentrations of dencichine decreased the levels of phospho-PI3K and phospho-AKT (Fig. 6) in LX-2 stimulated by PDGF-BB. Without affecting overall levels of the corresponding proteins PI3K and AKT (Fig. 4A).

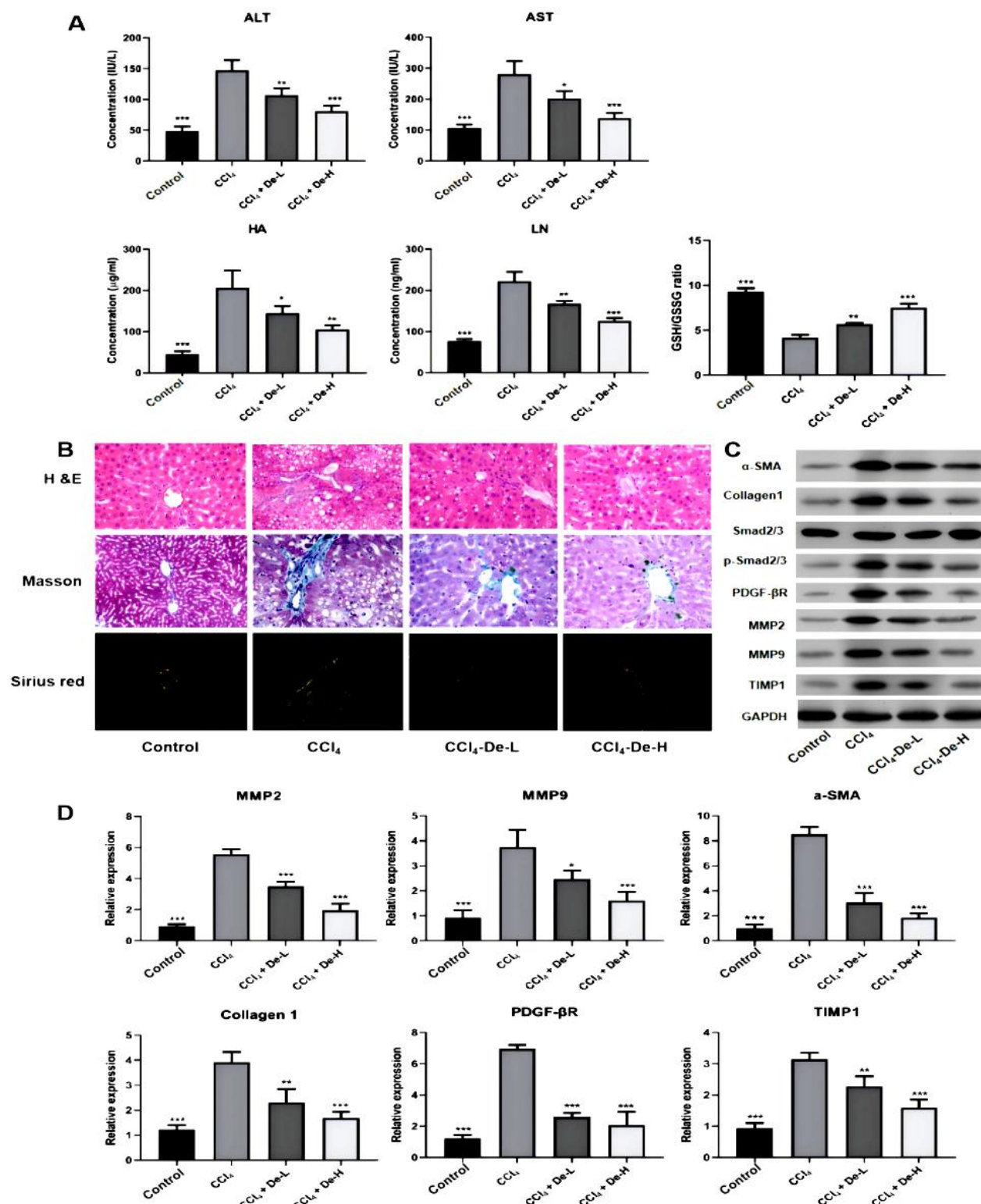


Fig. 1: Dencichine ameliorates mouse liver fibrosis induced by CCl₄. Induced the animal models of hepatic fibrosis caused by CCl₄, the mice were treated with different concentrations of dencichine, respectively. (A) The levels of ALT, AST, HA, LN content and GSH/GSSG level; (B) Histomorphology of liver tissues were evaluated by hematoxylin-eosin (H&E), Masson's trichrome, Sirius red methods; (C) Protein expression patterns were analyzed by western blot from liver samples; (D) Transcription levels of selected gene targets were quantified using quantitative RT-PCR. ($p < 0.05$ vs CCl₄; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $n=3$).

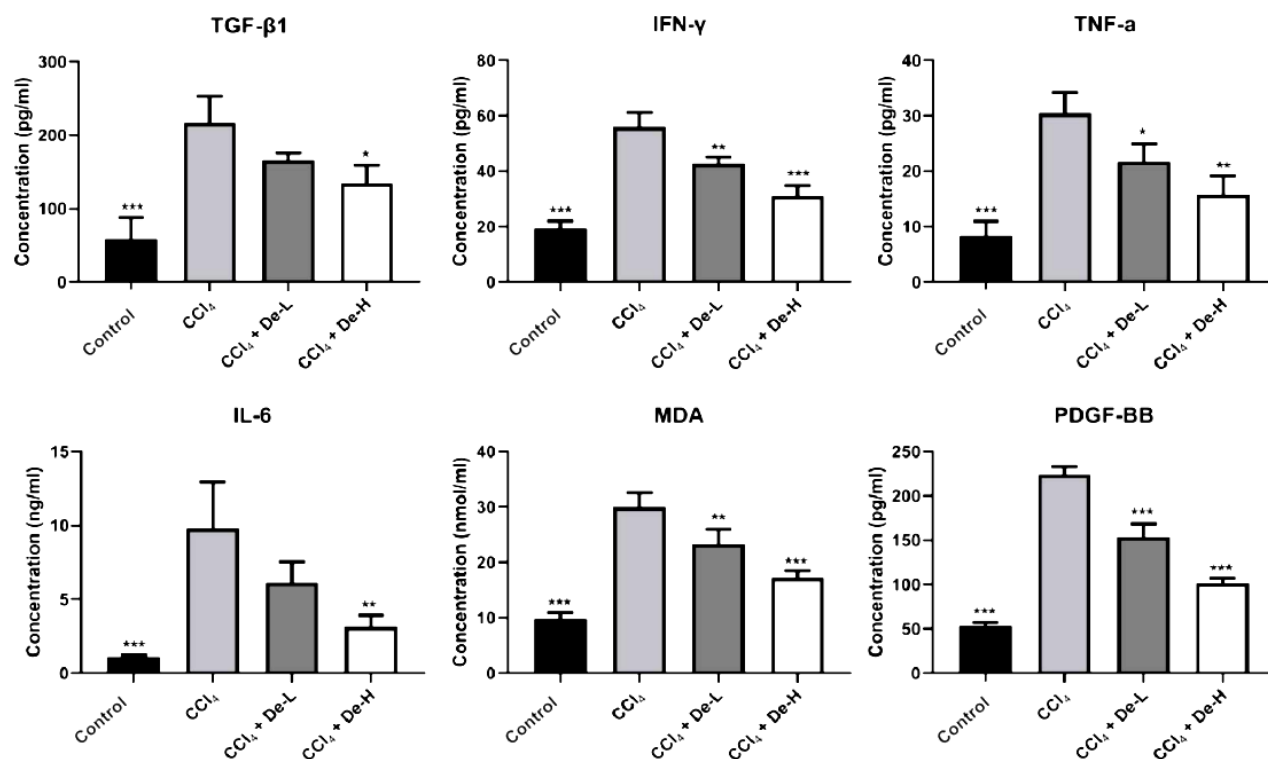


Fig. 2: ELISA detection of liver fibrosis-related protein concentrations in liver tissue Compared with the CCl₄ model group, the normal control group and dencichine-treated groups showed significantly reduced levels of TGF-β, IFN-γ, TNF-α, IL-6, MDA and PDGF-BB (*P < 0.05, **P < 0.01, ***P < 0.001, n=3).

Importantly, concomitant application of LY294002, a selective inhibitor for the PI3K cascade, significantly diminished dencichine mediated inhibition in α-SMA and Col1a1 expression (Fig. CyclinD1, CyclinE1, MMP2 and MMP9 proteins (Fig. 4B). These results were further confirmed by RT-PCR analysis of corresponding genes (Fig. 4C).

DISCUSSION

Liver fibrosis is one kind of chronic liver disease caused by long-term injury to the liver tissue. It mainly forms through the sustained activation and hyperproliferation of HSCs: which ultimately results in the pathological deposition of extracellular matrix (ECM) materials (Palomer *et al.*, 2025). In this study, we explored the pharmacological actions of Dencichine, one of the major active ingredients isolated from *Panax notoginseng*, using experimental models of liver fibrosis and clarified their mechanism of action at the molecular level. Our key finding is that Dencichine prevents or reduces the progression of fibrosis in animal and cell systems, via mainly inhibiting PI3K/AKT signaling leading to reduced proliferation/migration in HSCs.

In our animal experiments, we have provided definitive proof that administering dencichine can cure liver

fibrosis. In an animal model of chemical-induced liver fibrosis (CCl₄-induced), we found that dencichine treatment significantly improved liver function, reflected in decreased levels of ALT, AST, HA and LN. The treatment drug significantly reduced collagen deposition, as evidenced by a lower Sirius Red-stained area (Fig. 3). In particular, molecular studies showed that dencichine inhibited the expression levels of key fibrosis markers in a dose-dependent manner, including α-SMA and Collagen I. They are also good biological markers: alpha smooth muscle actin (α-SMA) indicates the activation of hepatic stellate cells, while Collagen I is the principal extracellular matrix component present in a fibrotic tissue (Zhang *et al.*, 2022, Wang *et al.*, 2024). This data strongly suggest that dencichine acts therapeutically by selectively targeting and modulating the pathologic activation of hepatic stellate cells, the main cellular mediators of liver fibrosis.

To better understand the effect of dencichine on HSCs, in vitro studies were carried out using a human HSC cell line (LX-2). The PI3K/AKT pathway is one of the key pathways that regulate multiple cellular events, such as survival, growth, energy usage and mobility and is of especial significance for promoting HSC survival during activation and persistence (Shamsan *et al.*, 2024, Zhang *et al.*, 2023, Chen *et al.*, 2022).

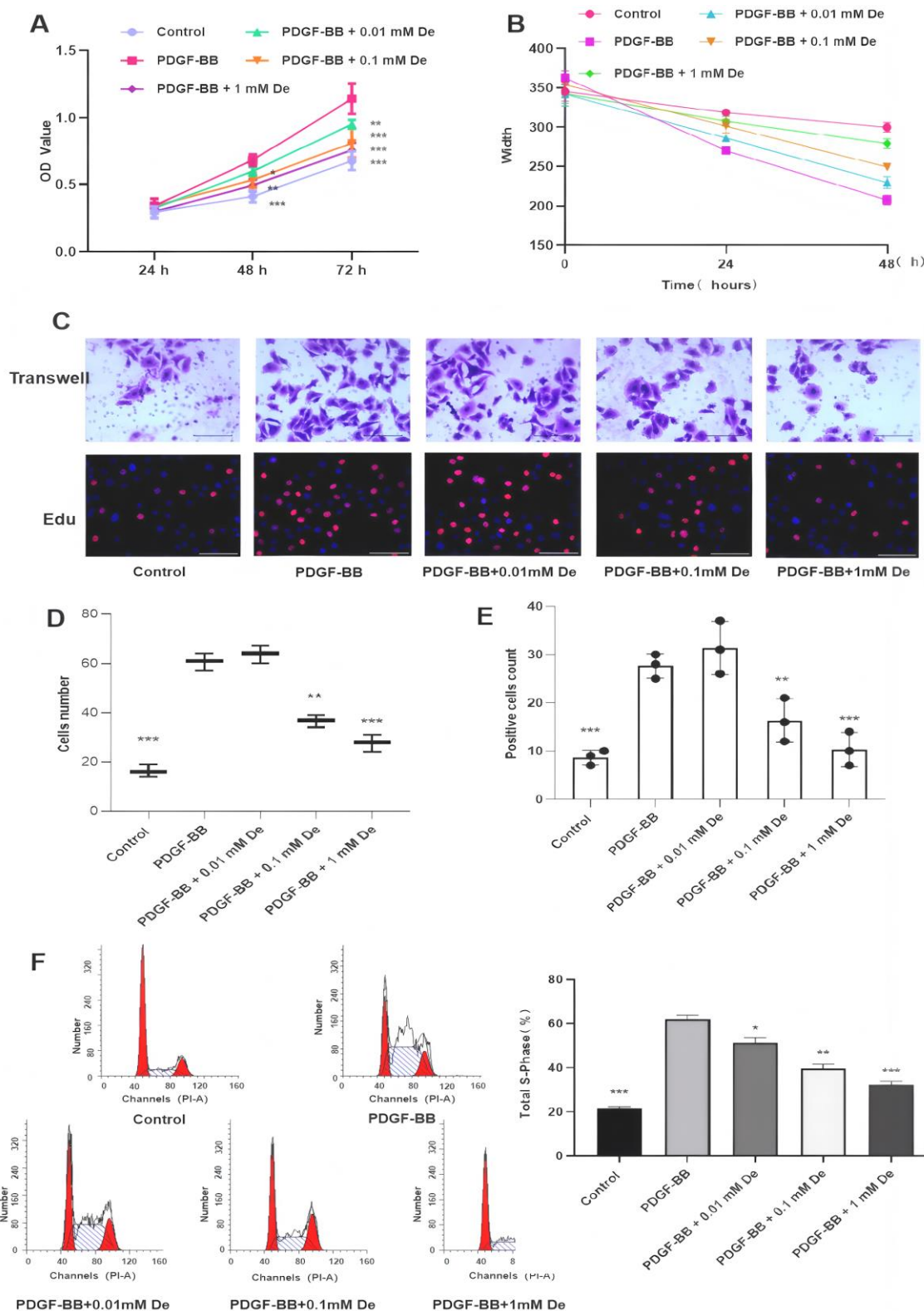


Fig. 3: The effect of dencichine on activated hepatic stellate cell induced by PDGF-BB. (A) Cell proliferation is detected with CCK-8 kit in PDGF-BB-activated hepatic stellate cells after treatment with dencichine; (B) Cell invasion is detected with scratch test; (C) Typical figures of Transwell and Edu experiments; (D) Quantitative statistical analysis for cell invasion according to Transwell experiment. (E-F) Cellular multiplication was determined through Edu testing and flow cytometric analysis, respectively. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $n=3$)

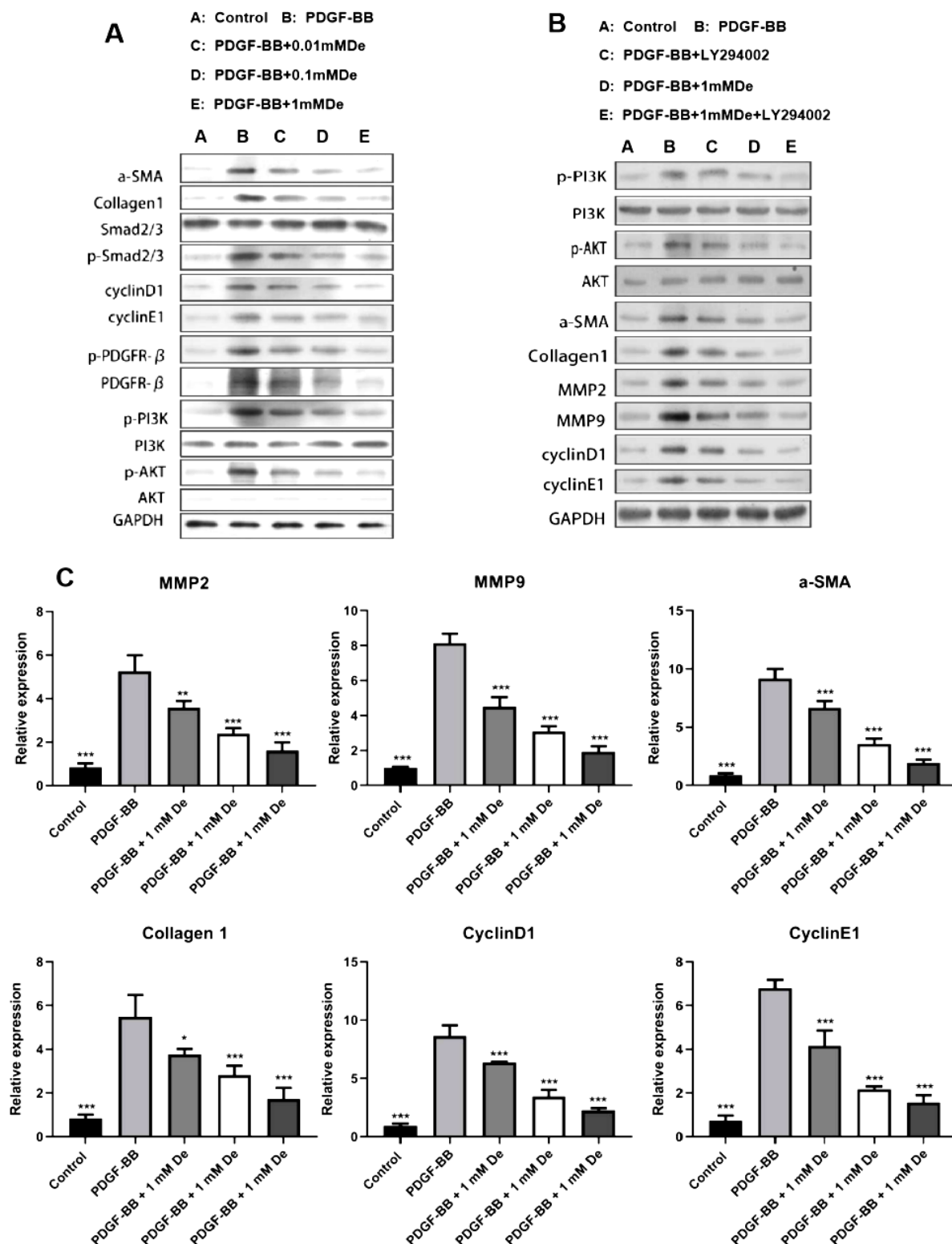


Fig. 4: Dencichine alleviates liver fibrosis through the PI3K/AKT pathway in a cellular model. (A) Western blot analysis of PI3K, AKT, Smad2/3, PDGFR- β protein expression and phosphorylation levels, as well as related functional proteins, in PDGF-BB-activated hepatic stellate cells treated with dencichine; (B) Protein expression and phosphorylation levels of PI3K and AKT, along with related proteins, in hepatic stellate cells after inhibitor treatment; (C) mRNA expression levels of α -SMA, Col1a1, CyclinD1, CyclinE1, MMP2, and MMP9 detected by RT-PCR. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $n=3$)

As shown by the experiments, treatment with dencichine significantly reduced p-PI3K and p-AKT levels in LX-2 cells without affecting their total amounts, suggesting specific inhibition of pathway activity rather than protein production. To confirm a direct relationship between PI3K/AKT inhibition and the resulting antifibrotic effects, we used the pharmacological inhibitor LY294002 (Wang *et al.*, 2017). In these studies, it was demonstrated that the anti-fibrotic activity of dencichine is due to its ability to inhibit the PI3K and AKT pathways.

The existing studies mainly explore the therapeutic effects of Dencichine on hepatic fibrosis via different signal transduction pathways, such as the MAPK (Gong *et al.*, 2022) JAK2/STAT3 (Hui *et al.*, 2016) and TGF- β (Li *et al.*, 2022, Zhan *et al.*, 2016) pathways. Despite of the fact that all these studies have a common finding that bioactive components derived from *Panax notoginseng* possess antifibrotic effects, the present study has some new results. In particular, our work first reports a role for dencichine in hepatic fibrosis development and clarifies how it exerts its effects via the PI3K/AKT signaling cascade.

In conclusion, Dencichine treatment was associated with reduced phosphorylation of PI3K and AKT, suggesting that the PI3K/AKT signaling pathway may be involved in its anti-fibrotic effect. However, direct evidence of pathway dependence (e.g., using specific activators or genetic knockdown) is still needed. Interestingly, unlike general kinase inhibitors, the compound's toxicity was low across all tested concentrations, suggesting a good safety profile. Furthermore, dencichine's well-known anti-inflammatory effect may work in synergy to its anti-fibrotic activity. Further experiments should evaluate whether dencichine affects liver immunity, which could have secondary effects on hepatic stellate cells.

There are some restrictions for this work. In this work, we mainly focus on the CCl₄-induced model of toxicity, a well-known model but with nevertheless different pathophysiologic pathways than other forms of fibrosis caused by viral infection, the absence of a positive control drug and the qualitative nature of histopathological assessment. Broadening testing of dencichine's pharmacological activity in additional preclinical models (e.g., low choline diet, STAM procedures, or cholangiopathy techniques) may more clearly demonstrate its utility. Furthermore, although we focused on the first PI3K/AKT pathway, the precise modulation of downstream molecular mediators (mTOR, GSK-3 β and NF- κ B pathways) remains to be further investigated. In addition, more detailed PK studies, biodistribution studies and long-term toxicity evaluations of dencichine should be conducted in the near future for the development of this compound as a novel preclinical drug candidate. In conclusion, this study shows that dencichine has potent

antifibrotic activity against liver fibrosis via inhibition of the PI3K/AKT pathway in HSCs. This study provides some evidence supporting the use of dencichine as an alternative drug or therapy strategy against fibrosis development in chronic liver injury.

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Author's contributions

Longkuan Li: Designed the experiments and completed the initial manuscript writing; Huan Luo: Performed the animal experiments; Zeyun Liu: Conducted the cell experiments; Wei Zhang, Yingqiang Chen and Fuxue Meng: Data analysis, revised the manuscript and handled the submission. Li Xian Mo: Performed substantial work in manuscript revision at the later stage and data analysis. Jinyou Tian: Served as an expert consultant and conducted comprehensive revision of the manuscript during the post-writing revision phase.

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Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files. Raw data are available from the corresponding author upon reasonable request.

Ethical approval

The study was approved by the Ethics Committee of The Third Affiliated Hospital of Guizhou Medical University (No. 2022A03). This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest

All authors declare no conflicts of interest.

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