



Babol University  
Of Medical Sciences

IJMCM, Summer 2025, VOL 14, NO 3

International Journal of Molecular and Cellular Medicine

Journal homepage: [www.ijmcm.org](http://www.ijmcm.org)



ORIGINAL ARTICLE

## Jianpi-Qinghua Formula alleviates liver dysfunction by suppression of HBV replication in HepG2.2.15 cells

Jianhua Xue<sup>1,2#</sup> , Beifen Zhong<sup>3#</sup> , Lifeng Wan<sup>1</sup> , Xiaofeng Hu<sup>1</sup> , Yinhua Zhang<sup>1</sup> , Minfang Zhu<sup>4</sup> , Jianjie Chen<sup>5</sup> , Hui Zhao<sup>6\*</sup> , Xiangxiang Wu<sup>7\*</sup>

1. Department of Infectious Diseases, Hospital for Infectious Diseases of Pudong District, Shanghai, 201299, P.R. China.
2. Department of Infectious Diseases, Shache County People's Hospital, Shache 844700, P.R. China.
3. Urologic Center, Shanghai General Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, 200080, P.R. China.
4. Pharmacy Department, Hospital for Infectious Diseases of Pudong District, Shanghai, 201299, P.R. China.
5. Department of infectious diseases, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 201203, P.R. China.
6. Shanghai Pudong New Area Mental Health Center, Shanghai, 200124, P.R. China.
7. Department of Rheumatism and Immunology, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, 200437, P.R. China.

### ARTICLE INFO

**Received:** 2024/11/27  
**Revised:** 2025/07/7  
**Accepted:** 2025/08/15

# These authors contributed equally to this work

**\*Corresponding:**

Hui Zhao  
**Address:**  
Shanghai Pudong New Area  
Mental Health Center.  
**E-mail:**  
38999199@qq.com

**\*Co-corresponding:**

Xiangxiang Wu  
**Address:**  
Department of Rheumatism  
and Immunology, Yueyang  
Hospital of Integrated  
Traditional Chinese and  
Western Medicine Affiliated  
to Shanghai University of  
Traditional Chinese Medicine.  
**E-mail:**  
wuxiangxiang204@163.com

### ABSTRACT

Previous researches revealed that Jianpi-Qinghua Formula (JPQH) exhibited anti-cancer activity. However, the pharmacological effect of JPQH on HBV-positive hepatocellular carcinoma (HCC) remains unknown. Cisplatin chemotherapy serves as a major treatment for HCC. Therefore, the aim of this study was to investigate whether JPQH or in combination with cisplatin therapy could inhibit the proliferation of HepG2.2.15 cells and elucidate the underlying mechanisms associated with the effect of cisplatin on HepG2.2.15 cells. We found that JPQH alleviated carbon tetrachloride (CCl<sub>4</sub>)-induced liver fibrosis and hepatitis in vivo. In vitro experiments, cisplatin effectively enhanced the suppression of HepG2.2.15 HCC cell proliferation and decreased their migration capacity. Moreover, JPQH could reduce CCl<sub>4</sub>-induced AST and ALT levels, as well as serum markers for liver fibrosis (including PIIINP, C-IV, LN, HA levels) and hepatitis markers (including TBIL, IL6, and IL-1β levels). JPQH also induced cellular apoptosis by downregulating the expression levels of proteins such as Bcl-2 and IL-6. Additionally, JPQH could downregulate HBV expression. These findings collectively demonstrate that JPQH may inhibit the proliferation of HepG2.2.15 cells through suppression of HBV replication.

**Keywords:** Jianpi-Qinghua Formula; CCl<sub>4</sub>; liver fibrosis; apoptosis; HBV replication

**Cite this article:** Xue J, et al. Jianpi-Qinghua Formula alleviates liver dysfunction by suppression of HBV replication in HepG2.2.15 cells. International Journal of Molecular and Cellular Medicine. 2025; 14 (3): 3797-3804. DOI: 10.22088/IJMCM.BUMS.14.3.797



© The Author(s).

Publisher: Babol University of Medical Sciences

This work is published as an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by-nc/4>). Non-commercial uses of the work are permitted, provided the original work is properly cited.

## Introduction

Hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer globally, ranking as the second leading cause of cancer-related deaths (1). HBV, an orthohepadnavirus of the Hepadnaviridae family, primarily infects hepatocytes, leading to progressive liver pathology (2). HBV infection is a primary risk factor for the development of HCC (3). HBV infection triggers immune-mediated hepatocyte injury, which initiates a pathological cascade progressing from hepatic fibrosis through cirrhosis to HCC (4). Despite surgical resection and radiotherapy serving as major treatments for HCC, patient survival rates remain suboptimal (5). Cisplatin chemotherapy is a potent chemotherapeutic agent commonly employed in the treatment of various cancers, such as sarcomas, cervical cancer, small cell lung cancer and others (6).

Nonetheless, the administration of chemotherapy in cancer patients heightens the risk of HBV reactivation. Multiple clinical studies have documented instances of HBV reactivation in individuals with HCC undergoing chemotherapy regimens involving cisplatin (7). Notably, many HCC patients exhibit resistance to cisplatin, diminishing its cytotoxic impact on cancer cells and increasing the likelihood of recurrence (8). The precise mechanisms underlying HBV reactivation remain elusive. In recent years, the exploration of novel drugs or drug repurposing has emerged as a key research focus in the realm of HCC treatment (9). The therapeutic efficacy and underlying principle of these medications lie in effectively suppressing HBV replication to curtail virus proliferation, thereby impeding or delaying disease progression. Nevertheless, nearly one million individuals continue to face chronic hepatitis B-related complications and mortality (10).

Jianpi-Qinghua Formula (JPQH), a formulation with a longstanding history of over two decades, is well-known for its efficacy in addressing lipid metabolism, intestinal inflammation, obesity, and insulin resistance (11, 12). Recent studies have highlighted JPQH's superior therapeutic impact on lipid dysregulation and inflammation in non-alcoholic fatty liver disease (NAFLD) (13). However, its precise therapeutic mechanism in chronic hepatitis B treatment remains elusive. Additionally, research has indicated that cisplatin can enhance HBV replication by

activating the ROS/JNK pathway while inhibiting the Akt/mTOR pathway (14). Therefore, the search for drugs that can enhance the cytotoxic effects of cisplatin and inhibit HBV replication is crucial.

HBV-associated hepatocellular carcinoma (HCC) remains a therapeutic challenge due to the dual burden of viral persistence and chemotherapy resistance. While cisplatin is a first-line chemotherapeutic agent, its efficacy is often compromised by HBV reactivation and stroma-driven chemoresistance. Therefore, we proposed for the first time that JPQH could inhibit HBV replication and effectively solve the problem of virus persistence.

In this study, we exposed the HepG2.2.15 cell line, which harbors the complete HBV genome, to a combination treatment with JPQH and cisplatin. This investigation aimed to unravel the impacts of a core combination of traditional Chinese medicine granules on cisplatin-induced proliferation, apoptosis, migration, and HBV replication in HepG2.2.15 cells.

## Methods

### Reagents

The JPQH formula included *Astragalus membranaceus* (Fisch.) Bunge (15 g), *Codonopsis pilosula* (Franch.) Nannf. (15 g), *Scutellaria baicalensis* Georgi (9 g), *Coptis chinensis* Franch. (3 g), *Rhizoma dioscoreae* (15 g), *Polygonatum sibiricum* (15 g), *Radix Puerariae* (15 g) and *Euonymus alatus* (Thunb.) Sieb (15 g) (12). JPQH formula was provided by Lei-Yun-Shang Pharmacy (Shanghai, China). The following antibodies had been used in this study: anti-actin monoclonal (sc-8432, Santa Cruz); anti-IL-6 polyclonal (21865-1-AP, Proteintech, China); anti-Bcl-2 monoclonal (12789-1-AP, Proteintech, China). All test kits were purchased from the manufacturer (Beyotime Biotechnology, China).

### Animal Models

Male SPF rats at 8 weeks of age were obtained from Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China). To collectively demonstrate JPQH's dual antiviral and antifibrotic actions, we selected CCl<sub>4</sub>-olive oil (Sinopharm Co., Ltd, China) solution to induce hepatic fibrosis. A total of 32 rats were randomly allocated into four groups (n=8 per group) based on body weight: (1) Control group receiving standard chow diet without CCl<sub>4</sub> treatment; (2) CCl<sub>4</sub>

model group intraperitoneally injected with 50% CCl<sub>4</sub>-olive oil solution (1 mL/kg) every 3 days for 6 weeks; (3) CCl<sub>4</sub> + JPQH(L) group administered low-dose JPQH (10 g/kg/day) via oral gavage; and (4) CCl<sub>4</sub> + JPQH(H) group receiving high-dose JPQH (15 g/kg/day) orally. JPQH or vehicle (saline) administration began at the 3rd week of CCl<sub>4</sub> induction and continued for 4 weeks, with daily body weight measurements and clinical symptom monitoring throughout the study period.

All animals were subject to tissue collection under anesthesia by intraperitoneal injection of pentobarbital. Livers were collected from the animals. The samples were flushed with phosphate-buffered saline (PBS, pH 7.4) and one part was rapidly sliced on ice and fixed in 4% paraformaldehyde solution, and the other instantly frozen in liquid nitrogen and then stored at -80°C until subsequent analysis. Ethical approval for all animal experiments was obtained from the Ethics Committee of Hospital for Infectious Diseases of Pudong District, Shanghai, China.

### Cell culture

HepG2.2.15 was obtained from American Type Culture Collection (ATCC) and cultured with DMEM (HyClone, USA) with 10% (v/v) fetal bovine serum (Gibco, USA). All cells were incubated at 37°C with 5% CO<sub>2</sub>. The HepG2.2.15 cell line, which stably replicates HBV, was selected to model viral persistence in HCC, which is essential for evaluating JPQH's antiviral mechanism.

### Serum analysis

The serum samples were obtained from 32 rats (four groups, eight rats per group). Total Bilirubin (TBIL), Human Procollagen III N-Terminal Propeptide (PIIINP), Collagen Type IV (IV-C), Laminin (LN), Hyaluronic acid (HA), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels (IU/L) were measured according to the International Federation of Clinical Chemistry method, which measured ultraviolet without pyridoxal phosphate using a Gen5 analyzer 7900-210 (BioTek, Vermont, USA).

### Hematoxylin and Eosin staining

The liver specimens were fixed in 4% paraformaldehyde solution, embedded in paraffin and sectioned. Sections measuring 5 μm thick were

meticulously prepared and mounted onto charged slides. Hematoxylin and eosin (HE) staining was carried out according to the manufacturer's instruction (Beyotime biotechnology, China). The stained slides were examined using an optical microscope, and high-quality images were captured utilizing the ZEISS celldiscoverer 7 imaging system (ZEISS celldiscoverer 7, Germany).

### Flow Cytometry Analysis

HepG2.2.15 cells were subjected to different treatments: cisplatin (10 μg/mL) for 24h, JPQH (200 μg/mL) for 2h, and a combination of cisplatin (24h) and JPQH (2h). After treatment, cells were harvested with 0.25% trypsin-EDTA, washed twice with cold PBS, and resuspended in 1× Binding Buffer (Annexin V-FITC/PI Apoptosis Detection Kit, Beyotime biotechnology, China).

Cells were stained with 5 μL Annexin V-FITC and 10 μL PI in the dark at 25°C for 15 min, followed by addition of 400 μL Binding Buffer. Samples were immediately analyzed on a BD flow cytometer (BD Biosciences, San Jose, CA) with the following settings: Excitation/Emission: 488 nm (FITC), 561 nm (PI). Gating Strategy: FSC-A vs. SSC-A: Exclude debris and cell aggregates. FITC-A vs. PI-A: Quadrant analysis to distinguish populations: Viable cells: Annexin V<sup>-</sup>/PI<sup>-</sup> (Q4). Early apoptotic: Annexin V<sup>+</sup>/PI<sup>-</sup> (Q3). Late apoptotic/necrotic: Annexin V<sup>+</sup>/PI<sup>+</sup> (Q2). Necrotic debris: Annexin V<sup>-</sup>/PI<sup>+</sup> (Q1). Data Acquisition: 10,000 events per sample, analyzed using FlowJo v10.8.1.

### Western Blot

The liver specimens were harvested and lysed. Protein quantification was performed using a BCA protein assay kit (Beyotime Biotechnology, China) according to the manufacturer's instruction. Protein aliquots were separated on 10% denaturing and transferred onto nitrocellulose membrane. The membrane was blocked in 5% BSA, and Primary antibodies against anti-IL-6 polyclonal (21865-1-AP, Proteintech, China, 1:1000 dilution) and anti-Bcl-2 monoclonal (12789-1-AP, Proteintech, China, 1:1000 dilution) were incubated overnight at 4°C. Membranes were then probed with horseradish peroxidase (HRP)-conjugated secondary antibody (1:2000) for 1 hour at room temperature. Blots were normalized to anti-actin monoclonal (sc-8432, Santa Cruz, 1:5000) as a loading

control. Subsequently, the protein bands on the nitrocellulose membrane were visualized using chemiluminescence detection reagent with Amersham Imager 800 (GE, Shanghai, China).

### Cell migration assays

HepG2.2.15 cells (25,000 cells/well) were seeded in the upper chamber of a laminin-coated Transwell insert (pore size 8  $\mu$ m; Corning, Cat# 3470) without growth factors. Laminin is a major component of liver extracellular matrix (ECM) that mediates cell adhesion and migration through integrin signaling. Cells were allowed to migrate through the membrane coated with laminin (20  $\mu$ g/ml). Growth factor and serum attractants were placed in the bottom chamber to stimulate cell migration. After a 24-hour incubation period, the percentage of cell invasion was determined by quantifying the number of cells that successfully migrated through the membrane out of the total number of cells initially plated.

### Detection of HBV gene copy number

HBV gene copy number was determined through PCR analysis using the Roche LightCycler 480 System (Roche, Basel, Switzerland). HBV DNA levels were determined using absolute quantification via a standard curve. The standard pCH9/3091-HBV1.1, with a concentration of  $5.5 \times 10^{10}$  copies/ $\mu$ L, served as the quantification reference and quantitative standard. The experiment included 5 dilution gradients. PCR amplification was performed for 39 cycles, and a PCR reaction curve was plotted using Ct value as the vertical axis and the log value of the standard copy number as the horizontal axis. PCR reactions were carried out with specific primers designed for HBV DNA amplification:

Forward primer:  
5'CCTAGTAGTCAGTTATGTCAAC-3' and  
reverse primer:  
5'-TCTATAAGCTGGAGGAGTGCGA-3'(15). HBV gene copy number detection was performed on the Hepg2.2.15 cells.

### Statistical Analysis

Data are presented as means  $\pm$  SD of three independent experiments. The results were analyzed using Student's t-test and one-way analysis of variance using SPSS 20.0 software (SPSS Inc., Chicago, IL).

## Results

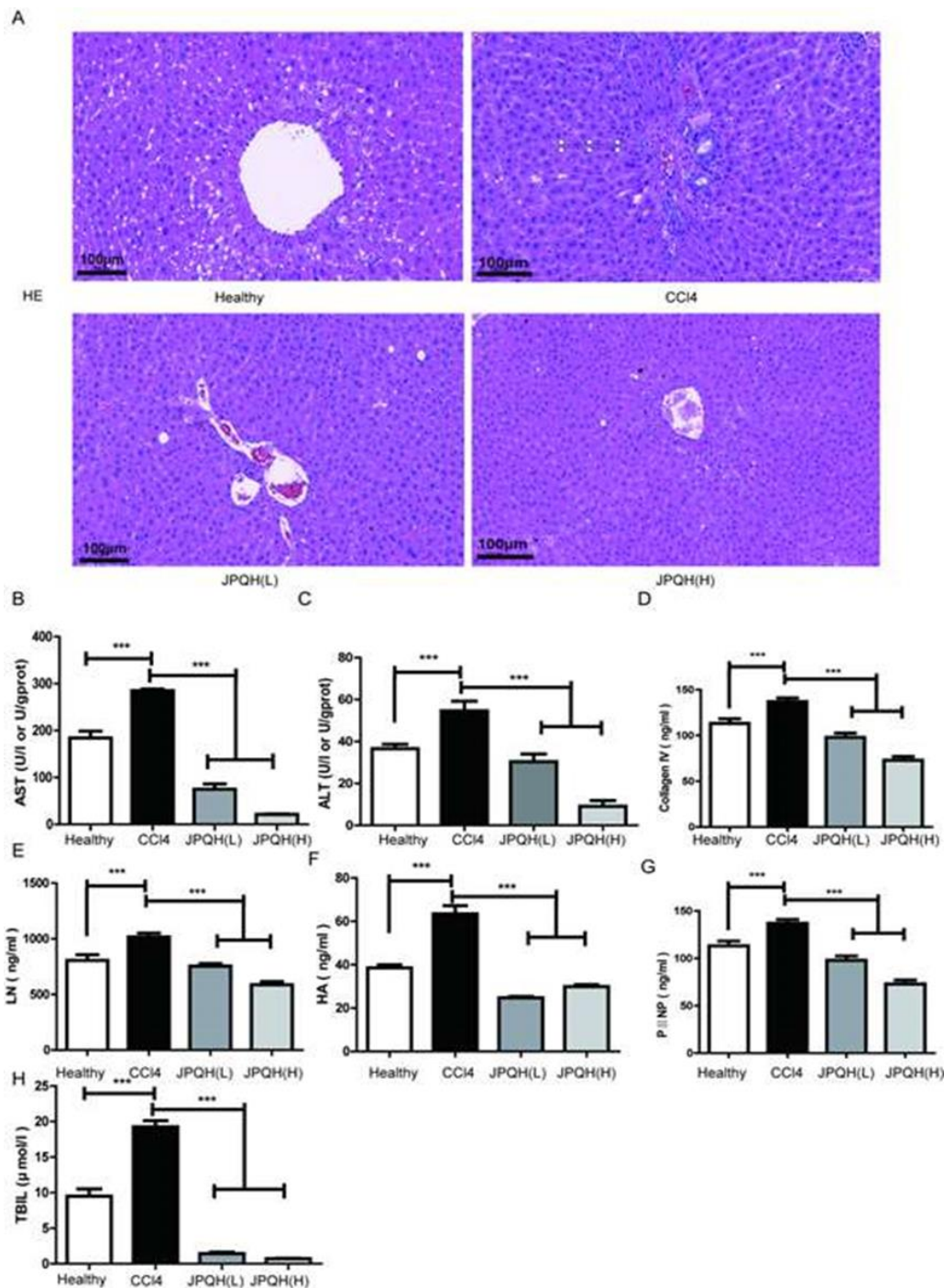
### JPQH Alleviated CCl4-induced Liver Fibrosis in Rats

To evaluate the anti-fibrotic efficacy of JPQH, histopathological and biochemical analyses were performed on liver tissues from four experimental cohorts (n=8/group): (1) Healthy controls (untreated); (2) CCl4-induced fibrosis model (50% CCl4-olive oil, 1 mL/kg, triweekly for 6 weeks); (3) CCl4 + JPQH(L) (10 g/kg/day oral gavage); (4) CCl4 + JPQH(H) (15 g/kg/day oral gavage). HE staining revealed that CCl4 treatment induced severe collagen deposition, with fibrotic areas occupying  $18.7 \pm 3.2\%$  of the liver parenchyma compared to  $4.1 \pm 0.9\%$  in healthy controls ( $p < 0.001$ ).

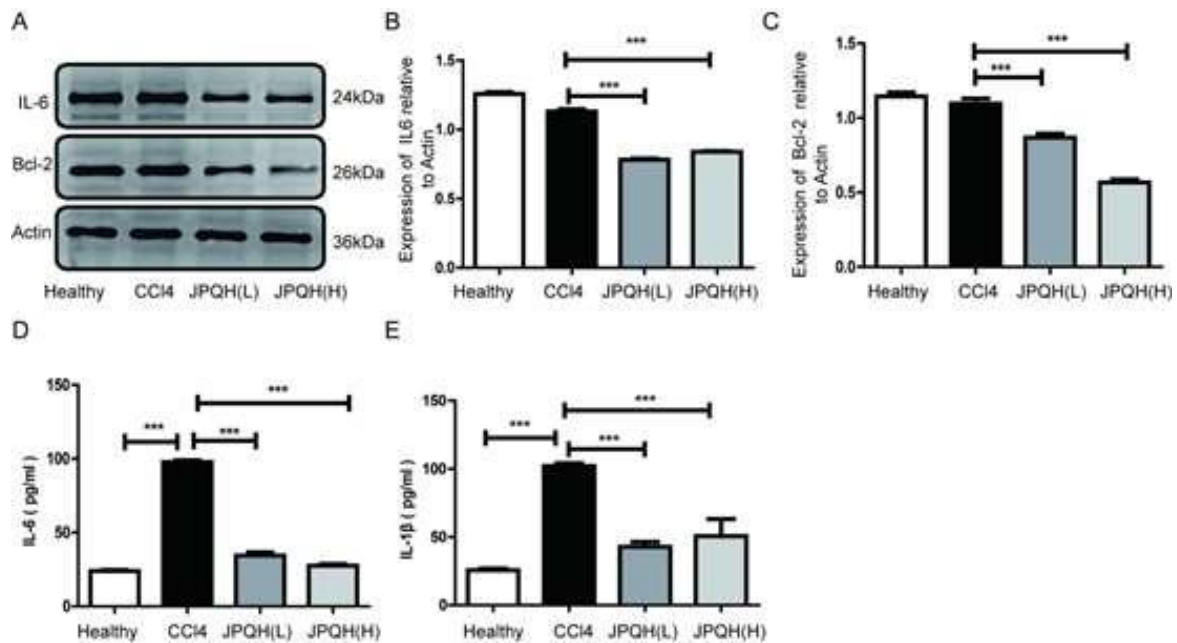
JPQH intervention dose-dependently attenuated fibrogenesis, of which fibrotic areas decreased to  $5.4 \pm 2.1\%$  in the JPQH(L) group ( $p = 0.003$  vs. CCl4 group) and  $1.8 \pm 1.5\%$  in the JPQH(H) group ( $p < 0.001$  vs. CCl4 group) (Figure. 1.A). Furthermore, compared to the Healthy group, the CCl4 group exhibited significantly higher mean AST and ALT levels, which were rescued in the JPQH(L) and JPQH(H) intervention groups (Figure. 1.B-C) ( $p < 0.001$ ). Serum markers for liver fibrosis, including PIIINP, C-IV, LN, HA, and the hepatitis marker TBIL were measured. The CCl4 group showed significantly higher levels compared to the Healthy group, which were reduced in the JPQH(L) and JPQH(H) intervention groups (Figure. 1.D-H) ( $p < 0.001$ ). These findings suggest the successful establishment of a CCl4-induced liver fibrosis model and demonstrate the efficacy of JPQH in reducing fibrosis in the rat model.

### Inhibition of Inflammation by JPQH *in Vivo*

The data presented strongly suggest that JPQH effectively inhibited CCl4-induced liver fibrosis and hepatitis. To further explore this, we evaluated the levels of Bcl-2 and IL6 in the liver using Western blot analysis, and IL6 and IL1 $\beta$  levels in the serum following JPQH treatment. In the CCl4 group, western blot analysis demonstrated that JPQH treatment significantly reduced Bcl-2 expression and IL-6 levels ( $p < 0.05$ , normalized to  $\beta$ -actin, n=3 independent experiments) (Figure. 2.A). Quantitative analysis further confirmed a significant decrease post-JPQH treatment (Figure. 2.B-C) ( $p < 0.001$ ).



**Figure 1. JPQH alleviates CCI4-induced liver injury *in vivo*.** (A) Pathological section analysis of H&E from the healthy, CCI4, JPQH (L) and JPQH (H) groups. The representative fields were captured from the liver tissue slides. (B-C) The serum levels of AST and ALT were determined using commercial reagent kits. The values are represented as means  $\pm$  SEMs and expressed in U/l of sera. Two-tailed t test, \*\*\*P < 0.001. (D) The serum levels of TBIL were measured. The values are represented as means  $\pm$  SEMs and expressed in  $\mu$ mol/l of sera. Two-tailed t test, \*\*\*P < 0.001. (E-H) The serum levels of Collagen IV, PIIINP, HA and LN were assessed using reagent kits. The values are represented as means  $\pm$  SEMs and expressed in ng/ml of sera. Two-tailed t test, \*\*\*P < 0.001.



**Figure 2. JPQH suppresses CCl<sub>4</sub>-induced inflammation *in vivo*.** (A) The liver samples were examined by Western blot to determine protein abundance. (B-C) IL-6 and Bcl2 were quantified, and the mean values were depicted in the bar graph beside the image. The data in bar Figure represents mean  $\pm$  SD (n = 3). Two tailed t test, \*\*\* P<0.001. The serum levels of IL-6 and IL-1 $\beta$  were measured. The values are presented as means  $\pm$  SEMs and expressed in ng/ml of sera. Two-tailed t test, \*\*\* P<0.001.

Furthermore, we examined the levels of IL6 and IL1 $\beta$  in the serum. The results revealed a significant increase in IL6 and IL1 $\beta$  levels in the CCl<sub>4</sub> group compared to the Healthy group, which were effectively normalized in the JPQH(L) and JPQH(H) intervention groups (Figure. 2D-E) ( $p < 0.001$ ). These findings provide compelling evidence that JPQH has the ability to inhibit hepatitis and alleviate liver damage induced by CCl<sub>4</sub>. The observed decrease in IL6 expression in the liver, along with the restoration of IL6 and IL1 $\beta$  levels in the serum, underscores the hepatoprotective effects of JPQH. These results highlight the potential therapeutic value of JPQH in combating liver fibrosis and inflammation. Further research is warranted to elucidate the specific mechanisms through which JPQH exerts its protective effects and to explore its clinical applications in the management of liver diseases

### JPQH Increased Anticancer Activity of Cisplatin in HepG2.2.15 Cell Lines

Cisplatin is the primary therapy for HCC accompanied by inflammation and apoptosis (16). In our investigation of the mechanism of JPQH in combination with cisplatin for HCC treatment, we

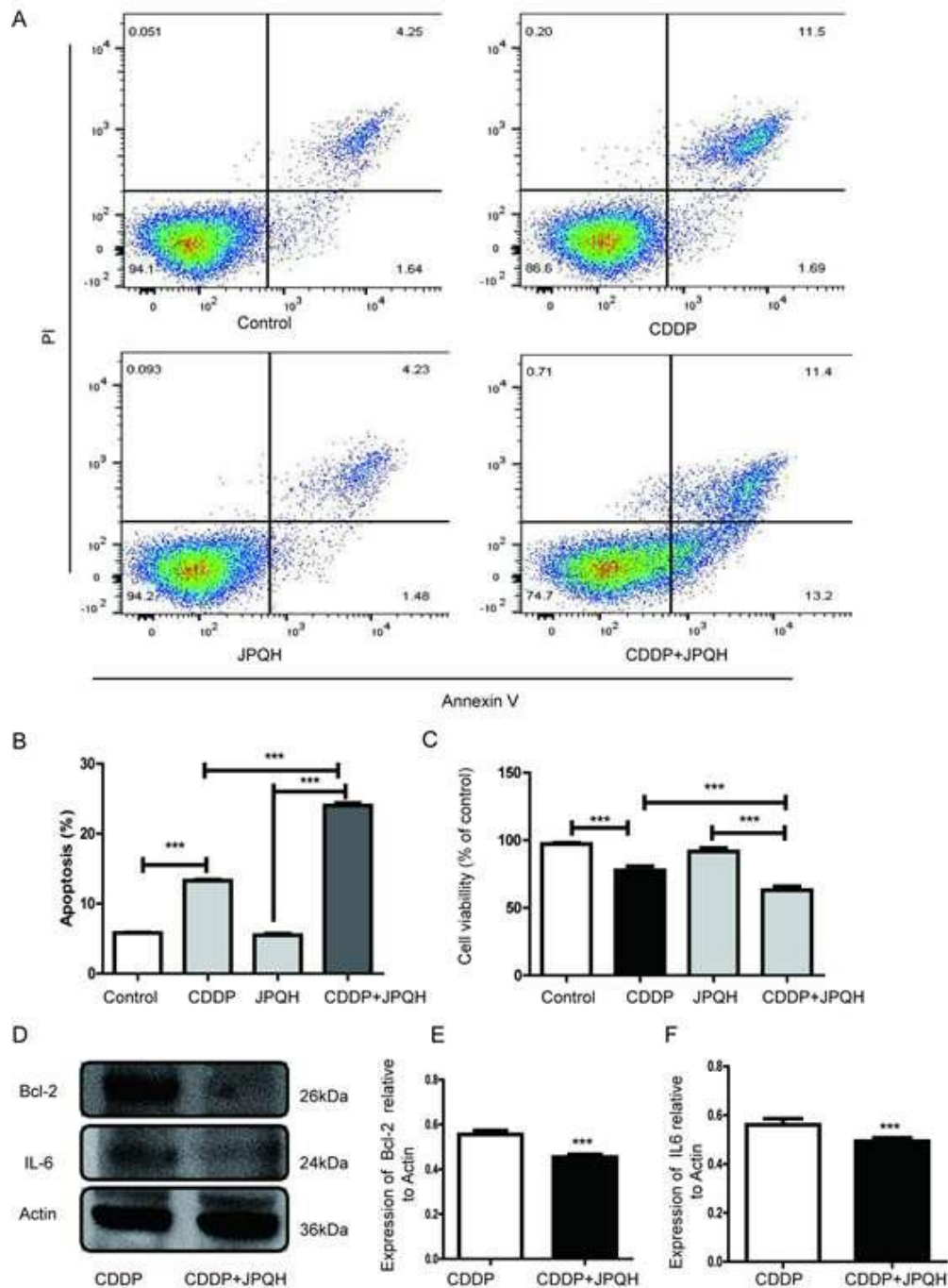
utilized the HepG2.2.15 cell line and divided the cells into four groups: Control group, Cisplatin group, JPQH group, and JPQH + Cisplatin group. We evaluated the apoptosis rate in each group, which revealed 5.89% apoptosis rate in the Control group, 13.19% in the cisplatin group, 5.71% in the JPQH group, and 24.6% in the JPQH + Cisplatin group.

Notably, the JPQH + Cisplatin combination significantly increased apoptosis in HepG2.2.15 cells compared to the Cisplatin group (Figure. 3.A-B) (\*\* $p < 0.001$ ). Moreover, we observed a consistent reduction in cell viability in the JPQH + Cisplatin group compared to the cisplatin group (Figure. 3.C) (\*\* $p < 0.001$ ). These findings suggest that the combination of JPQH with cisplatin enhances apoptosis in HepG2.2.15 cells and reduces cell viability, potentially indicating a synergistic effect in combating HCC.

JPQH effectively inhibited CCl<sub>4</sub>-induced hepatitis. To investigate this possibility, we evaluated the levels of Bcl-2 and IL6 using Western blot analysis. Cisplatin and JPQH + Cisplatin groups in HepG2.2.15 cells (Figure. 3.D). Quantitative analysis further confirmed a significant decrease following JPQH treatment (Figure. 3.E-F). Further exploration of the

underlying mechanisms driving these effects could offer valuable insights for optimizing therapeutic strategies for HCC treatment. The enhanced anticancer

activity observed with the combination of JPQH and cisplatin underscores the potential of this combination therapy in the management of HCC.

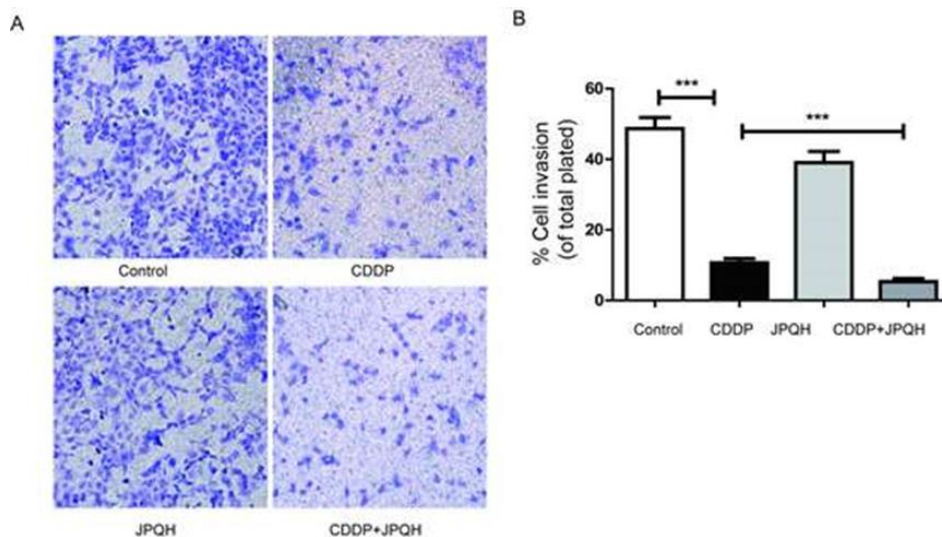


**Figure 3.** Effect of JPQH on anticancer activity of cisplatin in Hep2. 2.15 cells. (A) Apoptosis was detected by flow cytometry after treatment. The histograms showed the percentage of apoptotic cells. (B-C) Cell viability was determined by CCK-8. The data in bar graph represent mean  $\pm$  SD (n = 3). Two-tailed t test, \*\*\* P<0.001. The Hep2. 2.15 cells were examined by Western blot to determine protein abundance (D-F). Quantification of IL-6 and Bcl-2, with mean values depicted in the bar graph alongside the respective images. The data in bar graph represent mean  $\pm$  SD (n = 3). Two-tailed t test, \*\*\* P<0.001.

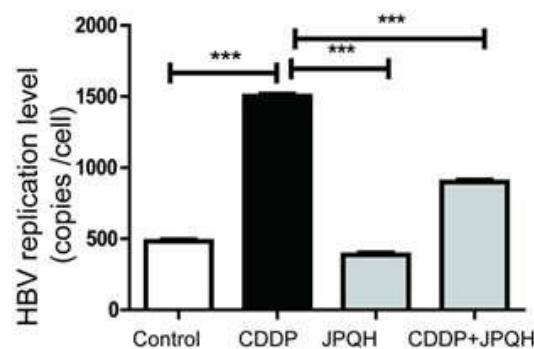
### JPQH Inhibited Cisplatin-Derived HBV Upregulation and Cell Migration in HepG2.2.15 Cell Lines

The HepG2.2.15 cell line, derived from the HCC cell line HepG2, is characterized by stable transfection with HBV expression (17). In our study, we investigated the HBV DNA copy number and cell migration capabilities of these cells. Our results demonstrate that the cisplatin treatment group exhibited a significant decrease in cell migration compared to the control group. Furthermore, the combination treatment group of JPQH + Cisplatin showed a notable reduction in cell migration compared to the cisplatin group (Figure. 4.A-B) (\*\* $p < 0.001$ ). Consistently, we observed a significant increase in HBV expression in the cisplatin

treatment group compared to the control group. However, the JPQH + Cisplatin combination treatment group exhibited a reduction in HBV expression compared to the cisplatin group (Figure. 5). These findings suggest that JPQH effectively inhibits cisplatin-induced HBV upregulation and cell migration in HepG2.2.15 cell lines. The combination of JPQH with cisplatin not only reduces cell migration but also mitigates the increase in HBV expression induced by cisplatin. This highlights the potential of JPQH as a therapeutic agent to counteract the effects of cisplatin on HBV upregulation and cell migration in the context of HCC (Figure. 6). Understanding the mechanism of action of JPQH is crucial for driving the development of novel therapies for HCC.



**Figure 4.** JPQH collaborates with cisplatin suppresses cell invasion in HepG2.2.15 cells. JPQH to inhibit cell invasion (A) Transwell invasion assays showed a decreased percentage of cell invasion in Cisplatin-treated and Cisplatin+JPQH-treated cells. Representative images of the Transwell chamber membranes are provided. (B) The percentage of four groups (including Control, Cisplatin, JPQH, Cisplatin+JPQH groups;  $n = 3$  wells). Bars represent mean  $\pm$  SEM, Two-tailed  $t$  test, \*\*\*  $P < 0.001$ .



**Figure 5.** Detection of HBV gene copy number by PCR. The data in bar graph represent mean  $\pm$  SD, Two-tailed  $t$  test, \*\*\*  $P < 0.001$ .

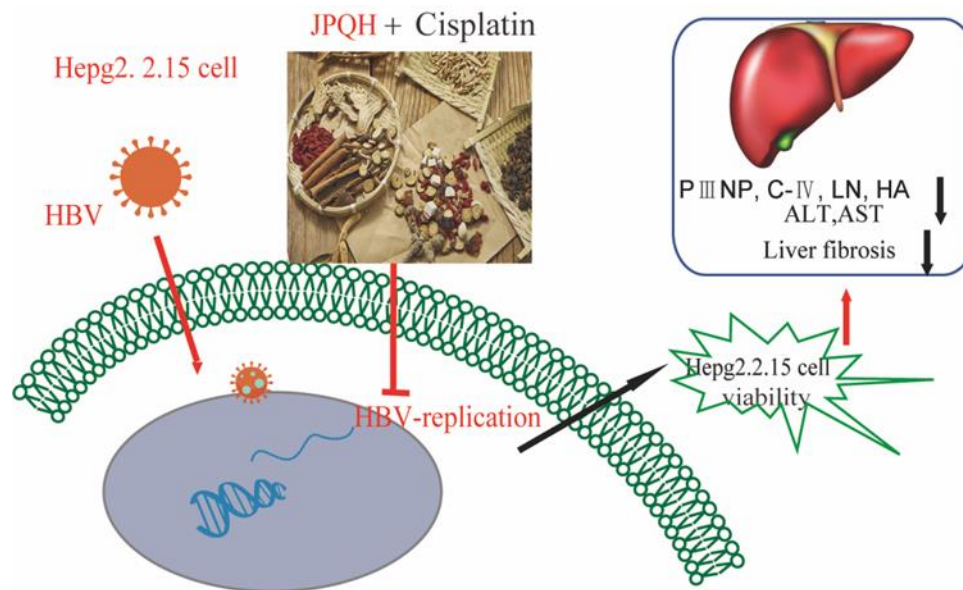


Figure 6. Schematic diagram illustrating the mechanism of JPQH in treating HBV-associated HCC and hepatic fibrosis.

## Discussion

Human hepatocellular carcinoma (HCC) mostly develops as a complication of fibrosis or cirrhosis (17). CCl<sub>4</sub>-induced liver fibrosis has been widely used as a model to simulate the pathogenesis of human liver fibrosis (18). Liver cell injury accounts for increased inflammatory cell infiltration and the release of pro-inflammatory cytokines, which are major inducers of liver fibrosis (19). In our study, by measuring the levels of ALT and AST, the liver injury markers, we found that JPQH reduced CCl<sub>4</sub>-induced liver injury (Figure. 1.B-C). Serum markers for liver fibrosis, including PIIINP, C-IV, LN, HA, and the hepatitis marker TBIL were also measured. The results demonstrated a promising therapeutic potential of JPQH in alleviating liver fibrosis and hepatitis and enhancing the anticancer activity of cisplatin in HCC.

The effects of JPQH on liver fibrosis and inflammation highlight its hepatoprotective properties, suggesting a multifaceted role in combating liver diseases. The significant reduction in fibrotic areas, inflammatory cell infiltration, and restoration of liver function markers in the JPQH intervention groups underscore the efficacy of JPQH in ameliorating liver cell injury. Moreover, our findings revealed that JPQH treatment effectively inhibited fibrosis by modulating the levels of Bcl-2 and IL6 in the liver, the key factor involved in the formation of fibrosis and pro-fibrotic cytokines, as well as IL6 and IL1 $\beta$  in the serum.

The restoration of cytokine levels and reduction in pro-inflammatory markers indicated the anti-inflammatory and hepatoprotective effects of JPQH in mitigating liver injury. Interestingly, the network pharmacology suggests that the active ingredients-targets of JPQH are FASN and NF $\kappa$ B1A, which are related to inflammation (12). Given that these cytokines are the inducers of pro-fibrotic pathogenesis, we speculated that JPQH might inhibit the hepatic fibrogenesis partly through the suppression of inflammatory signaling. These results suggested that JPQH might serve as a valuable therapeutic agent for liver diseases characterized by fibrosis and inflammation.

The increased sensitivity to cisplatin after HBV infection may involve multiple mechanisms (20). Our study demonstrated that the combination of JPQH with cisplatin enhanced the anticancer activity of cisplatin in HepG2.2.15 cells. In addition, JPQH treatment effectively inhibited fibrosis by modulating the levels of Bcl-2 and IL6 in HepG2.2.15 cells. The synergistic effect observed in terms of increased apoptosis and reduced cell viability in the combination treatment group underscores the potential of JPQH to augment the efficacy of conventional chemotherapeutic agents in combating HCC. Furthermore, the inhibition of cell migration and modulation of HBV expression by the JPQH + Cisplatin combination treatment hint at additional mechanisms through which JPQH may exert its anticancer effects.

Cisplatin chemotherapy exacerbates HBV-associated HCC progression by inducing CTLA-4-mediated CD8<sup>+</sup> T cell exhaustion, which promotes HBV reactivation and activates the IL-6/STAT3/Bcl-2 oncogenic axis. Mechanistically, HBV reactivation upregulates IL-6 transcription, driving STAT3 phosphorylation and subsequent overexpression of the anti-apoptotic protein Bcl-2, ultimately inducing chemoresistance. JPQH treatment effectively counteracts this process by downregulating IL-6 and Bcl-2 expression in HepG2.2.15 cells, thereby suppressing fibrotic progression and restoring cisplatin sensitivity. The therapeutic efficacy of JPQH stems from its dual mechanism of action: (1) inhibiting HBV cccDNA transcription and (2) blocking NF- $\kappa$ B nuclear translocation. This synergistic intervention simultaneously targets viral persistence and stromal remodeling, presenting a novel therapeutic strategy for HBV-related HCC.

In China, HBV has a vital contribution to HCC development. The HepG2.2.15 cells, derived from HepG2 by stable transfection with HBV genome (21), provide a model to study the interplay between JPQH, cisplatin, and HBV expression in HCC. Our findings suggested that cisplatin treatment could effectively reduce cell migration and apoptosis, and the combination of JPQH with cisplatin further enhanced this effect. Clinical studies have reported that HBV reactivation occurs in patients with HCC undergoing chemotherapy by using cisplatin, leading to a sharp increase in HBV DNA levels. Consistent with these findings, our study also observed an increase in HBV DNA levels with cisplatin treatment. However, the JPQH + cisplatin combination treatment group exhibited a reduction in HBV expression compared to the cisplatin group (Figure.5).

Additionally, the modulation of HBV expression by these treatments indicates a potential link between HBV DNA levels and the observed effects on cell migration and apoptosis. Further exploration of the molecular mechanisms underlying the synergistic effects of JPQH and cisplatin, as well as the impact of JPQH on HBV-related processes, could provide valuable insights for developing novel therapeutic strategies for HCC management. Future studies focusing on elucidating the specific signaling pathways and molecular targets involved in the interactions between JPQH, cisplatin, and HBV are warranted to

fully harness the therapeutic potential of JPQH in the context of liver diseases, particularly HCC.

In conclusion, our study highlights the multifaceted therapeutic effects of JPQH in mitigating liver fibrosis, inhibiting hepatitis, and enhancing the anticancer activity of cisplatin in HCC. A comprehensive understanding of these effects could pave the way for the development of novel treatment approaches targeting liver diseases, with JPQH emerging as a promising candidate for further clinical investigation.

## Acknowledgements

This study was approved by the ethics committee with the ethical code of 2021-001 (Hospital for Infectious Diseases of Pudong District, Shanghai). This study was supported by the Cultivation of Academic Leaders in Pudong New Area (grant no. PWRd2021-20), Kashi Regional Science and Technology Bureau Project (KS2019071), Clinical Traditional Chinese Medicine Characteristic Discipline in Pudong New Area (PDZY-2018-0607), Science and Technology Development Fund of Shanghai Pudong New Area (PKJ2023-Y04).

## References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394-424.
2. Li X, Pan E, Zhu J, et al. Cisplatin Enhances Hepatitis B Virus Replication and PGC-1 $\alpha$  Expression through Endoplasmic Reticulum Stress. *Sci Rep.* 2018;8(1):3496.
3. Shi YW, Yang RX, Fan JG. Chronic hepatitis B infection with concomitant hepatic steatosis: Current evidence and opinion. *World J Gastroenterol.* 2021;27(26):3971-83.
4. Wandera BO, Onyango DM, Musyoki SK. Hepatitis B virus genetic multiplicity and the associated HBV lamivudine resistance mutations in HBV/HIV co-infection in Western Kenya: A review article. *Infect Genet Evol.* 2022;98:105197.
5. Yang Z, Liu S, Hu L, et al. Stereotactic body radiotherapy is an alternative to radiofrequency

- ablation for single HCC  $\leq 5.0$  cm. *JHEP Rep.* 2024;6(10):101151.
6. Xu Y, Wang C, Li Z. A new strategy of promoting cisplatin chemotherapeutic efficiency by targeting endoplasmic reticulum stress. *Mol Clin Oncol.* 2014;2(1):3-7.
  7. Jang JW, Kwon JH, You CR, et al. Risk of HBV reactivation according to viral status and treatment intensity in patients with hepatocellular carcinoma. *Antivir Ther.* 2011;16(7):969-77.
  8. Crissien AM, Frenette C. Current management of hepatocellular carcinoma. *Gastroenterol Hepatol (N Y).* 2014;10(3):153-61.
  9. Zou J, Ma Q, Sun R, et al. Dihydroartemisinin inhibits HepG2.2.15 proliferation by inducing cellular senescence and autophagy. *BMB Rep.* 2019;52(8):520-4.
  10. Bollerup S, Hallager S, Baek O, et al. Implementation of treatment recommendations for chronic hepatitis B in patients attending specialized hospital care in Denmark - a region wide study. *Scand J Gastroenterol.* 2020;55(7):843-7.
  11. Liu Y, Han X, Cai M, et al. Jianpi Qinghua Formula alleviates insulin resistance via restraining of MAPK pathway to suppress inflammation of the small intestine in DIO mice. *BMC Complement Med Ther.* 2022;22(1):129.
  12. Wang L, Chen QG, Lu H. Jianpi Qinghua Formula Alleviates Diabetic Myocardial Injury Through Inhibiting JunB/c-Fos Expression. *Curr Med Sci.* 2024;44(1):144-55.
  13. Tian J, Cai M, Jin S, et al. JianPi-QingHua formula attenuates nonalcoholic fatty liver disease by regulating the AMPK/SIRT1/NF-kappaB pathway in high-fat-diet-fed C57BL/6 mice. *Pharm Biol.* 2023;61(1):647-56.
  14. Chen X, Hu Y, Zhang W, et al. Cisplatin induces autophagy to enhance hepatitis B virus replication via activation of ROS/JNK and inhibition of the Akt/mTOR pathway. *Free Radic Biol Med.* 2019;131:225-36.
  15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the  $2^{-\Delta\Delta C(T)}$  Method. *Methods.* 2001;25(4):402-8.
  16. Tasneem S, Liu B, Li B, et al. Molecular pharmacology of inflammation: Medicinal plants as anti-inflammatory agents. *Pharmacol Res.* 2019;139:126-40.
  17. Uehara T, Pogribny IP, Rusyn I. The DEN and CCl4 -Induced Mouse Model of Fibrosis and Inflammation-Associated Hepatocellular Carcinoma. *Curr Protoc Pharmacol.* 2014;66:14 30 1-14 30 10.
  18. Forbes SJ, Newsome PN. Liver regeneration - mechanisms and models to clinical application. *Nat Rev Gastroenterol Hepatol.* 2016;13(8):473-85.
  19. Zhang L, Liu C, Yin L, et al. Mangiferin relieves CCl4-induced liver fibrosis in mice. *Sci Rep.* 2023;13(1):4172.
  20. Zhang X, Zhang R, Yang H, et al. Hepatitis B virus enhances cisplatin-induced hepatotoxicity via a mechanism involving suppression of glucose-regulated protein of 78 Kda. *Chem Biol Interact.* 2016;254:45-53.
  21. Sells MA, Chen ML, Acs G. Production of hepatitis B virus particles in Hep G2 cells transfected with cloned hepatitis B virus DNA. *Proc Natl Acad Sci U S A.* 1987;84(4):1005-9.