

Network Pharmacology Prediction and Experiment Validation to Discover the Mechanism of Sophora Japonica Linn against Liver Cancer

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Abstract

Sophora Japonica Linn, a medicinal and food-homologous plant, is commonly used to resist bacterial, inflammatory, tumor, and other effects. This study aimed to elucidate the multi-target mechanism of action of *Sophora japonica* Linn on liver cancer through network pharmacological analysis and verify its effect through biological experiments. The network pharmacology and molecular docking results showed that there were 152 interactivity targets between *Sophora japonica* Linn and liver cancer, which were mainly enriched in various biological processes through the PI3K/AKT and MAPK signaling pathways. In vitro biological experiments showed that isorhamnetin and quercetin, the main active components of *Sophora japonica* Linn, had significant inhibitory effect on liver cancer cells HepG2. In addition, the expression of AKT and MEK proteins was downregulated, which proved that both isorhamnetin and quercetin promoted apoptosis by activating the PI3K/AKT and MAPK signaling pathways. In summary, our findings clarify the inhibitory effect of the active ingredients of *Sophora japonica* Linn against HepG2 cells and provide inspiration for its clinical application in the treatment of liver cancer.

1. Introduction

Liver cancer is the most common cause of cancer-related death worldwide, the fifth most common cancer in the world, and the only cancer with a percentage increase in incidence each year ^[1]. Current treatment options for liver cancer include natural compounds, chemotherapeutic drugs, immunotherapies, and new drug delivery methods ^[2]. The long-term use of chemotherapy drugs, such as sorafenib, has additional problems such as toxicity or ineffectiveness of the drug. In addition, immunotherapy and drug delivery therapy are not significantly effective in improving the outcomes of this devastating disease ^[3]. Therefore, new technologies or methods urgently need to be developed for the prevention and treatment of liver cancer. Natural compounds can provide better patient outcomes, reduced systemic toxicity, and fewer side effects.

Sophora Japonica Linn (SJL) is widely distributed in temperate regions worldwide as a wild and cultivated tree species. Modern pharmacological studies have shown that the efficacy of SJL is closely related to its main component, flavonoids ^[4]. Many clinical studies have shown that flavonoids are common bioactive components in most plants, with many properties, including anti-inflammatory activity, enzyme inhibition, anti-bacterial activity, anti-cancer, anti-allergic activity, anti-oxidant activity, vasoactivity, and cytotoxic anti-tumor activity ^[5–6]. Through in-depth research on the flavonoids in SJL, it was found that SJL contains many pharmacological compounds such as rutin, narcine, nicotinin, kaempferol, quercetin, isorhamnetin, and genistein ^[7–8].

Network pharmacology is a discipline that uses bioinformatics, that is, the use of biological and computer science knowledge to predict the pharmacological effects related to traditional Chinese medicine ^[9]. Network pharmacology can provide evidence at the molecular level and a new treatment method for traditional Chinese medicine research. Since Hopkins proposed the concept of network

pharmacology in 2007, research on Chinese compound medicine, Chinese herbal medicine, and monomer drugs has become a hot topic in domestic Chinese medicine research^[10]. However, network pharmacology methods rely heavily on computer science, which is a prediction mechanism based on big data, and there is no accurate research data to prove the absolute correctness of its pharmacology. After prediction through network pharmacology, experiments can be designed according to the prediction results to avoid some detours^[11]. In this study, we established an "SJL-components-disease-pathways" network; based on the findings of network pharmacology, we obtained two key bioactive ingredients and three representative targets.

2. Materials and Methods

2.1 Screening the candidate compounds of SJL

All active ingredients of SJL are based on the pharmacological database and analysis platform (<https://tcmsp-e.com/>) of the Chinese medicine system^[12]. We screened available active ingredients based on oral bioavailability (OB) $\geq 30\%$ and druggability (DL) ≥ 0.18 index^[13].

2.2 Targets analysis of SJL and Liver Cancer

Candidate compound targets of SJL were screened using the TCMSP database. Subsequently, we switched the protein names of all targets to the corresponding gene names on the UniProt website (<https://www.uniprot.org/>)^[14]. Potential target genes associated with liver cancer were obtained from the GeneCards database^[15].

2.3 Venn analysis and PPI network construction

The Venny 2.1 online tool (<https://bioinfogp.cnb>) was used to analysis liver cancer targets with potential targets of SJL active ingredients^[16]. String database (<https://string-db.org/>) could be used to obtain PPI networks and exclude targets without correlation based on confidence. Cytoscape software (version 3.9.0) was used to build the PPI interactive network and visualize it^[17].

2.4 Enrichment analysis of biological functions

The DIVID (<https://david.ncifcrf.gov/>) database was used to analysis the potential core targets of SJL-liver cancer interaction, and the relevant data of GO enrichment analysis and KEGG signaling pathway were downloaded from the analysis results. The bioinformatics website (<http://www.bioinformatics.com.cn/>) was used to create online graphs^[18].

2.5 Molecular Docking

Molecular docking was performed on the PDB database, and the protein was preprocessed to remove water molecules, add hydrogen bonds, remove the original ligand, and then saved as a PDBQT format file^[19]. AutoDock calculates the binding energy based on various interactions between amino acid residues

between the two. The binding energy less than -4.25 , -5.0 , and -7.0 kcal/mol indicate a certain, good, or strong binding ability between the ligand and receptor, respectively^[20]. The lower the binding energy, the more stable the binding between the receptor and ligand. Finally, the molecular docking results were converted into PDBQT format using OpenBabel GUI and imported into Paymol software for visualization display^[21].

2.6 Cell culture

The human liver cancer cell line HepG2 was purchased from the Cell Bank of the Typical Culture Preservation Committee of the Chinese Academy of Sciences and stored in our laboratory's cell seed bank. HepG2 cells were cultured in DMEM with 10% fetal bovine serum and incubated at 37°C and 5% CO₂.

2.7 Cell proliferation analysis by MTT method

The effects of SJL activity compounds isorhamnetin (Selleck, S9111) and quercetin (Selleck, S2391) on HepG2 cells were detected using the MTT assay. Briefly, cells were seeded in 96-well plates at a density of 5,000 cells/well and cultured at 37°C for 16 h. Cells were then treated with different concentrations of isorhamnetin (0, 20, 40, and 60 μ M) and quercetin (0, 50, 75, and 100 μ M) for 24, 48, and 72 h, respectively. MTT solution was added and incubated at 37°C for 4 h. Absorbance at 490 nm was measured using a spectrophotometer.

2.8 Clone formation assay

HepG2 cells were seeded into 6-well plates at a density of 1000 cells/well and cultured with different concentrations of isorhamnetin (0, 20, 40, and 60 μ M) and quercetin (0, 50, 75, and 100 μ M), and the medium was changed every three days. After 14 days, cells were fixed with 4% paraformaldehyde and stained with crystal violet.

2.9 Wound healing assay

HepG2 cells were cultured in 6-well plates for 48 h at a density of 5×10^5 cells/well. These cells were then scratched vertically with a pipette tip to create an artificial wound. Media containing different concentrations of isorhamnetin (0, 20, 40, and 60 μ M) and quercetin (0, 50, 75, and 100 μ M) were added to each well. After 48 h, the cells were observed under a microscope. Data analysis was performed using ImageJ software.

2.10 Apoptosis and cell cycle detection

HepG2 cells were seeded into 60 mm culture dishes and cultured with isorhamnetin (0, 20, 40, and 60 μ M) and quercetin (0, 50, 75, and 100 μ M) for 2 days. The cells were collected and washed with phosphate-buffered saline (PBS). After centrifugation at 1,500 rpm for 5 min, the cells were resuspended in 195 μ L of Annexin V-FITC/PI labeling solution and incubated for 10–15 min. After centrifugation at 1,500 rpm for 5 min, the supernatant was removed and 500 μ L of fixative solution with 70% cold ethanol was added to the cells. After washing, 500 μ L of PI/RNaseA staining solution was added, and the cells

were protected from light at room temperature for 30–60 min. The cells were immediately analyzed using a flow cytometer.

2.11 Western blotting

The cells were lysed with RIPA for 30 min and then transferred to centrifuge tubes. After centrifugation at 12,000 rpm for 10 min, a BCA protein assay kit was used to quantify protein concentration. After separation by SDS-PAGE, 50 µg samples were transferred onto PVDF membranes, sealed with 5% skim milk powder for 1 h at room temperature, and washed with PBST solution. The rabbit anti-AKT (Beyotime Biotechnology, AF1777), MEK1 (Beyotime Biotechnology, AF1252), ERK1/2 (Beyotime Biotechnology, AF1051), p-AKT (Beyotime Biotechnology, AF5740), p-MEK1 (Beyotime Biotechnology, AF1786), p-ERK1/2 (Beyotime Biotechnology, AF1891) monoclonal antibodies, after incubation overnight at 4 °C, washing the film again, adding the corresponding secondary antibody. The gray values of each imaging protein band were analyzed by PS and ImageJ, and the changes in phosphorylated protein were judged based on the ratio of phosphorylated protein levels to total protein, β -actin served as an internal control.

2.12 Statistical analysis

Data are presented as the mean $\pm$ SD. The results were analyzed using GraphPad Prism 8.0 and SPSS 20.0. Quantitative data between groups were compared using Student's t-test and one-way ANOVA, and the difference was statistically significant at $p < 0.05$.

3. Results

3.1 The targets in SJL and Liver Cancer screening

Six bioactive compounds were identified in SJL by searching the TCMSP database and conducting ADME screening ($OB \geq 30\%$ and $DL \geq 0.18$)^[22]. The six compounds were quercetin, isorhamnetine, kaempferol, β -sitosterol, N-[6-(9-acridinamino)hexyl]benzamide, and quercetin-3'-methyl ether (Table 1).

Table 1
Bioactive Compounds of Sophora Japonica Linn

Mol ID	Molecule Name	MW	OB(%)	DL	CAS number	Pubchem Cid
MOL000354	isorhamnetin	316.28	49.60	0.31	480-19-3	5281654
MOL000422	kaempferol	286.25	41.88	0.24	520-18-3	5280863
MOL000358	beta-sitosterol	414.79	36.91	0.75	83-46-5	222284
MOL005935	N-[6-(9-acridinylamino)hexyl]benzamide	397.56	41.70	0.78	N/A	146515
MOL005940	quercetin-3'-methyl ether	316.28	46.44	0.30	N/A	N/A
MOL000098	quercetin	302.25	46.43	0.28	117-39-5	5280343

As shown in Fig. 1A, the proteins corresponding to the six bioactive components of SJL were screened from the TCMSP database, and we removed duplicate values and transferred protein names to gene symbols in the Uniport database, and 171 predicted targets of SJL were obtained (**Supplementary Table S1**). A total of 6098 related target genes for liver cancer were collected from GeneCards (relevance score ≥ 5 , **Supplementary Table S2**). Among 171 SJL-related targets and 6098 liver cancer-related targets, there were 152 overlapping identification targets (**Supplementary Table S3**), which are considered pivotal targets for subsequent research.

3.2 The construction of SJL-Liver Cancer-related PPI network and C-D-T network

As presented in Fig. 1B, the PPI network, which contains 151 nodes and 1112 edges, was constructed using the STRING database, in which nodes represent proteins and edges represent protein–protein interactions. To further visualize and analyze protein-protein interactions, the retrieved PPI data were subsequently imported into Cytoscape 3.9.0 to construct a visualization PPI network with 142 nodes and 1112 edges (Fig. 1C). The association between the six active compounds in SJL and 171 target genes for SJL was visualized in the C-D-T network, which contained 177 nodes and 251 edges. In the C-D-T network, orange elliptical nodes symbolize the target genes, green diamond-shaped nodes represent the bioactive components of SJL, and rose-red inverted triangles indicate SJL (Fig. 1D).

3.3 GO and KEGG pathway enrichment analysis

We performed GO and KEGG pathway enrichment analyses to study the function and enrichment pathways of the potential anti-liver cancer genes of SJL. The results showed that the targets of SJL in the treatment of liver cancer were mainly enriched in positive regulation of transcription, DNA templating (GO:0045893), positive regulation of gene expression (GO:0010628) and other biological processes,

negative regulation of the apoptotic process (GO:0043066), extracellular space (GO:0005615), macromolecular complex (GO:0032991) and other cellular components, extracellular region (GO:0005576), enzyme binding (GO:0019899), protein binding (GO:0005515), identical protein binding (GO:0042802), and other cellular components (Fig. 2A, 2B, 2C).

There were 172 SJL-liver cancer-related pathways with statistical significance, among them, the top 30 significant enrichment potential pathways with the highest gene counts are presented in a bar plot diagram (Fig. 2D), illustrating that SJL plays an important role in the treatment of liver cancer through multiple targets and multiple pathways. The five pathways with high gene numbers were the cancer signaling pathway (n = 66), lipid and atherosclerotic signaling pathway (n = 39), PI3K signaling pathway (n = 33), AGE-RAGE signaling pathway (n = 30), and MAPK signaling pathway (n = 30), which may be the key pathways for the anti-liver cancer effect of SJL.

3.4 Molecular docking analysis isorhamnetin and quercetin interaction with AKT1, TP53 and TNF

Three candidate target proteins, AKT1 (PDB ID: 4GV1), TP53 (PDB ID: 4IBQ), and TNF (PDB ID: 6O0Z), were molecularly docked with two candidate bioactive compounds: isorhamnetin and quercetin. The results were visualized using AutoDock and PyMOL, showing that isorhamnetin and quercetin could interact with AKT1, TP53, and TNF, respectively ^[23]. As shown in Fig. 3A, isorhamnetin interacted with HIS-220 and ARG-220 in AKT1 through one hydrogen bond and MET-458 through two hydrogen bonds, respectively. Isorhamnetin interact with THR-150, PRO-152, THR-155 and ARG-202 in TP53. Isorhamnetin could interact with LEU-120, SER-60, GLN-61, and TYR-119 by forming one hydrogen bond in TNF. The structure of quercetin interacts with LEU-347, GLU-341, ARG-243, and TYR-350 to form one hydrogen bond, one hydrogen bond, two hydrogen bonds, and two hydrogen bonds in AKT1. Quercetin interacts with LEU-137, PRO-152, and TYR-220 to form a hydrogen bond in TP53. Quercetin interacted with SER-99, TYR-115, LYS-112, GLN-102, and ARG-103 in TNF to form two hydrogen bonds, one hydrogen bond, one hydrogen bond, two hydrogen bonds, and one hydrogen bond in TNF, respectively (Fig. 3B).

3.5 Isorhamnetin and quercetin inhibited the proliferation and migration of HepG2 cells

Two active compounds, isorhamnetin and quercetin, were evaluated for their inhibition of cell proliferation of HepG2 cells by MTT and plate cloning experiments. As shown in Fig. 4A, isorhamnetin inhibits the proliferation of HepG2 cells in a concentration-dependent manner, and the half effective inhibitory concentration decreases over time, with an IC₅₀ value of 36.76 μ M at 48 h. The number of HepG2 cell clones gradually decreased with increasing isorhamnetin concentration, indicating that isorhamnetin inhibited the growth of HepG2 cells (Fig. 4B). As shown in Fig. 4C, with the decrease in quercetin concentration, the proliferation ability of HepG2 cells was weakened, and the 48 h IC₅₀ value of quercetin was 72.06 μ M. The inhibitory effect of quercetin on HepG2 cells was also confirmed by the colony formation assay, which indicated that quercetin could inhibit the growth of HepG2 cells with an

increase in quercetin concentration (Fig. 4D). As shown in Fig. 5A, 5B, we evaluated the migration ability of HepG2 cells treated with isorhamnetin and quercetin in a wound healing formation experiment and found that isorhamnetin and quercetin significantly inhibited the migration of HepG2 cells in a concentration-dependent manner.

3.6 Isorhamnetin and quercetin induced apoptosis and cycle arrest of HepG2 cells

We further evaluated the effects of isorhamnetin and quercetin on apoptosis and cell cycle distribution of HepG2 cells using propidium iodide staining followed by flow cytometry analysis. The apoptosis results were shown in Fig. 6A, high doses of isorhamnetin and quercetin increased the apoptosis rate of HepG2 cells compared to the control group, indicating that isorhamnetin and quercetin could induce apoptosis of HepG2 cells. As shown in Fig. 6B, isorhamnetin induced a remarkable decrease in the G1 phase cells of HepG2. Quercetin induced an accumulation of HepG2 cells in the G2 phase with a concomitant decrease in the population in the S phase. These results indicated that isorhamnetin and quercetin induced apoptosis and cell cycle arrest in HepG2 cells.

3.7 Isorhamnetin and quercetin inhibited the expression of PI3K/AKT/MAPK proteins in HepG2 cells

Bioinformatics analysis showed that AKT1 and MAPK were among the top ten core targets, and the PI3K/Akt and MAPK pathways were the most enriched signaling pathways in the treatment of liver cancer with SJL. Therefore, to further investigate the underlying mechanisms by which isorhamnetin and quercetin exhibit anti-liver cancer activity, we measured the protein expression in HepG2 cells of p-AKT, AKT, p-MEK1, MEK1, p-ERK1/2, and ERK1/2. As shown in Fig. 7A, the phosphorylation levels of AKT, MEK1, and ERK1/2 were significantly reduced in HepG2 cells induced by isorhamnetin compared to those in the control. The phosphorylation changes of AKT and MEK1 in quercetin-treated HepG2 cells were downregulated, and the expression of p-ERK1/2 was upregulated compared to the control (Fig. 7B).

SC79 is a unique AKT activator that is capable of increasing the activity of intracellular AKT under various physiological and pathological conditions ^[24]. Subsequently, we detected the corresponding changes in the expression levels of AKT, MEK1, ERK1/2, and the corresponding phosphorylated proteins after the use of SC79. As shown in Fig. 7C, the phosphorylation of AKT, MEK1, and ERK1/2 was upregulated in HepG2 cells induced by isorhamnetin after the use of SC79. After the use of the activator SC79, the expression of p-AKT, p-MEK1, and p-ERK1/2 in quercetin-induced HepG2 cells was upregulated (Fig. 7D). These results indicate that SC79 could activate PI3K/AKT/MAPK signaling pathways, and PI3K/AKT/MAPK signaling pathways play a crucial role in liver cancer with SJL.

4 Discussion

In recent years, several retrospective studies have shown that the combination of traditional Chinese medicine and liver cancer can improve the survival rate of patients with unresectable liver cancer ^[25]. The multi-component, multi-target, and multi-pathway of traditional Chinese medicine has become an important advantage in pharmaceutical research. However, this advantage also brings new difficulties in the study of the mechanism of action of drugs ^[26]. In this study, network pharmacology provided a research concept for the development of drugs for the treatment of liver cancer with SJL.

The active ingredients of SJL include phytosteroids, tannins, quercetin, kaempferol, isoflavones, isorhamnetin, genistein, rutin, and other flavonoids ^[27]. Modern pharmacological studies have shown that the active ingredient or crude extract of SJL has a wide range of pharmacological effects, such as cardiovascular, anti-inflammatory, anti-osteoporosis, anti-oxidant, anti-tumor, anti-bacterial, anti-viral, hemostatic, and anti-atherosclerotic effects ^[28]. Most of these effects were consistent with the results observed in popular medicine. Younsook Kim et al. reported that SJL buds provide a potential means for the treatment of celiac disease, and its therapeutic mechanism can be explained by the relationship between 11 major components and 13 CD-related genes ^[29]. Another research team studied the bioactive flavonoids in SJL, in which high doses of kaempferol inhibited the growth of HepG2 cells ^[30]. Liu et al. also predicted from network pharmacology and molecular docking that quercetin, the most important component of SJL, can inhibit the levels of inflammatory factors, phosphorylated c-Jun levels, and the PI3K-AKT signaling pathway in ulcerative colitis cells ^[31]. In this study, we found that SJL affects the proliferation, apoptosis, and migration of human HepG2 cells.

The main active ingredients of SJL screened through the network were quercetin and isorhamnetin. Mirazimi et al. reported that quercetin has a significant inhibitory effect on tumor progression through various mechanisms of action, including stimulation of cell cycle arrest and/or apoptosis, and its anti-oxidant properties ^[32]. Lu et al. showed that quercetin can reverse docetaxel resistance in prostate cancer through androgen receptors and the PI3K/AKT signaling pathway ^[33]. Moreover, recent studies have reported that quercetin can reduce tumor microenvironment components and can be used to inhibit the growth of hepatocellular carcinoma ^[34]. The results of this study showed that quercetin affected the proliferation, apoptosis, and migration of human HepG2 cells through the PI3K/AKT and MAPK signaling pathways. Recent studies have shown that isorhamnetin and its glycoside forms can exert a wide range of pharmacological effects and health benefits, including cardiovascular and cerebrovascular, neuroprotective, anti-inflammatory, kidney-protective, lung-protective, anti-osteoporosis, anti-oxidant, obesity prevention, and anti-tumor effects ^[35]. Du et al. reported that isorhamnetin may confer radiosensitivity to A549 cells by increasing IL-13 expression and inhibiting NF- κ B activation ^[36]. Shi et al. studied isorhamnetin, the active ingredient of corn whiskers, to inhibit the progression of gastric adenocarcinoma through the MAPK/mTOR signaling pathway ^[37]. Isorhamnetin has been shown to exhibit hepatoprotective effects by enhancing anti-oxidant defenses and reducing inflammation through a variety of signaling pathways, including inflammation ^[38]. In this study, isorhamnetin inhibited

HepG2 cell proliferation and induced apoptosis and migration by downregulating the phosphorylation of AKT, ERK1/2, and MEK1.

This study is the first to combine network pharmacology with experimental verification to study the mechanism of action of SJL against liver cancer. In summary, through network pharmacology and experimental verification, it was verified that isorhamnetin and quercetin could inhibit the PI3K/AKT and MAPK/ERK pathways, leading to apoptosis of hepatoma cell HepG2 and inhibiting their proliferation and migration. This study provides a theoretical basis for the clinical treatment of liver cancer using SJL.

Declarations

Funding Declaration

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Data Availability Statement

The authors confirm that the data supporting the findings of this study are available in the article and its supplementary materials. The data of this manuscript is stored in Science Data Bank, the link is as follows, <https://www.scidb.cn/en/s/IF3eYf>; CSTR link is <https://cstr.cn/31253.11.sciencedb.18397>; DOI link is <https://doi.org/10.57760/sciencedb.18397>.

Author' Contributions

Conceptualization: Y.R., W.S. Experiment operation: Yun. Liang., Y. F., M. Z., S.Z. Data curation and formal analysis: Y.R., T.Z., W.S. Funding acquisition: Y.R., T.Z. Methodology: Y.R., X.X., Q.C., Yue. Liu. Paper writing r: Yun. Liang., Y.R. All authors have given approval to the final version of the manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethics Statement

This study was approved by the Ethics Committees of Henan University of Urban Construction.

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Figures

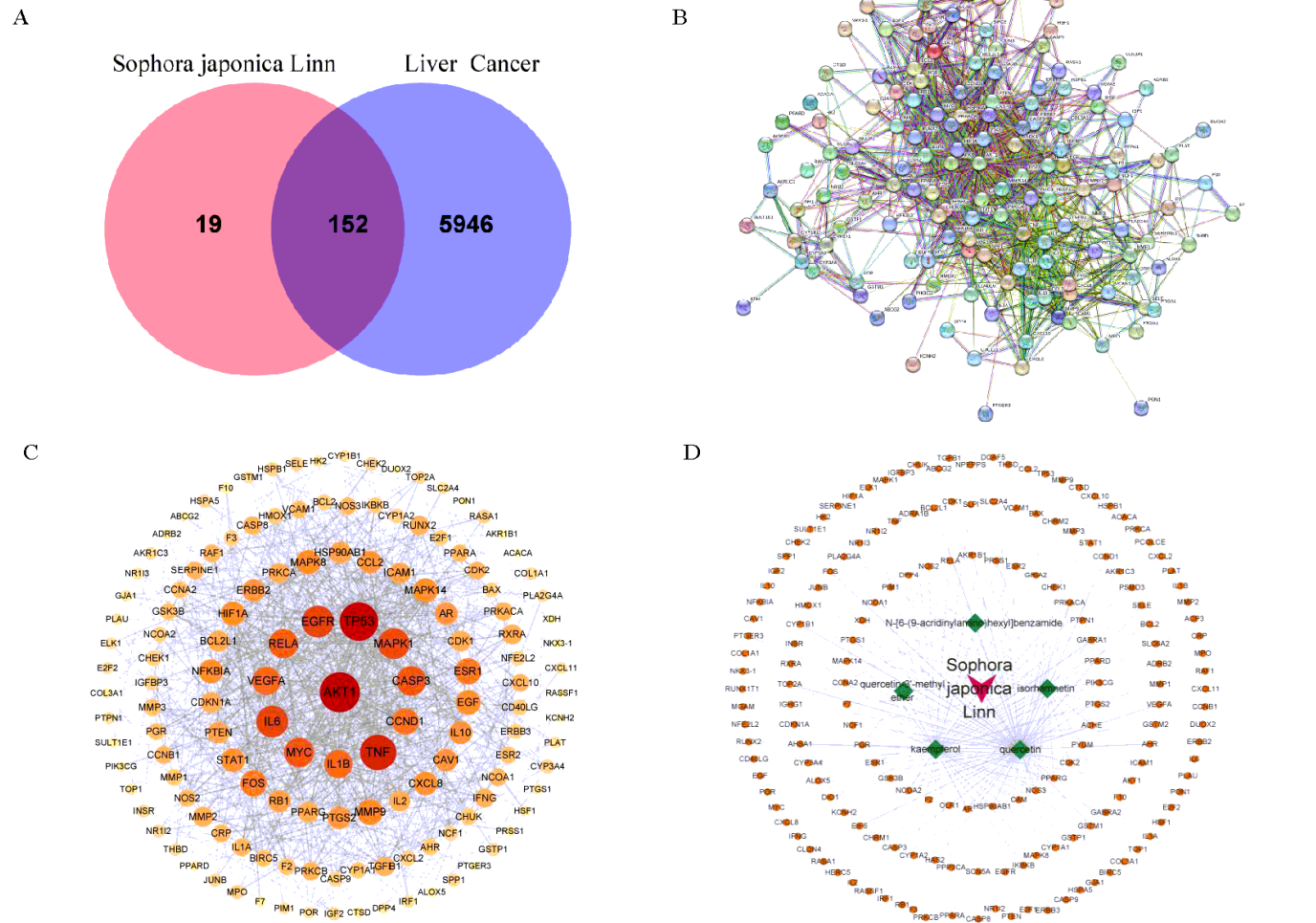


Figure 1

The venn diagram and PPI network of SJL-Liver Cancer. (A) The venn diagram showed that there were 152 common targets of the SJL and liver cancer. (B) The interactive PPI network obtained from STRING database with the minimum required interaction score set to 0.700. It comprised 151 nodes and 1112 edges, each node represents relevant targets, and edges stand for protein-protein associations. (C) PPI network imported cytoscape 3.9.0 to obtain a visual network diagram. (D) C-D-T network of SJL. The orange color in the elliptical node represents the target genes, the green diamond-shaped dots represent the bioactive compounds of SJL, the rose inverted triangle represents the locust herbs.

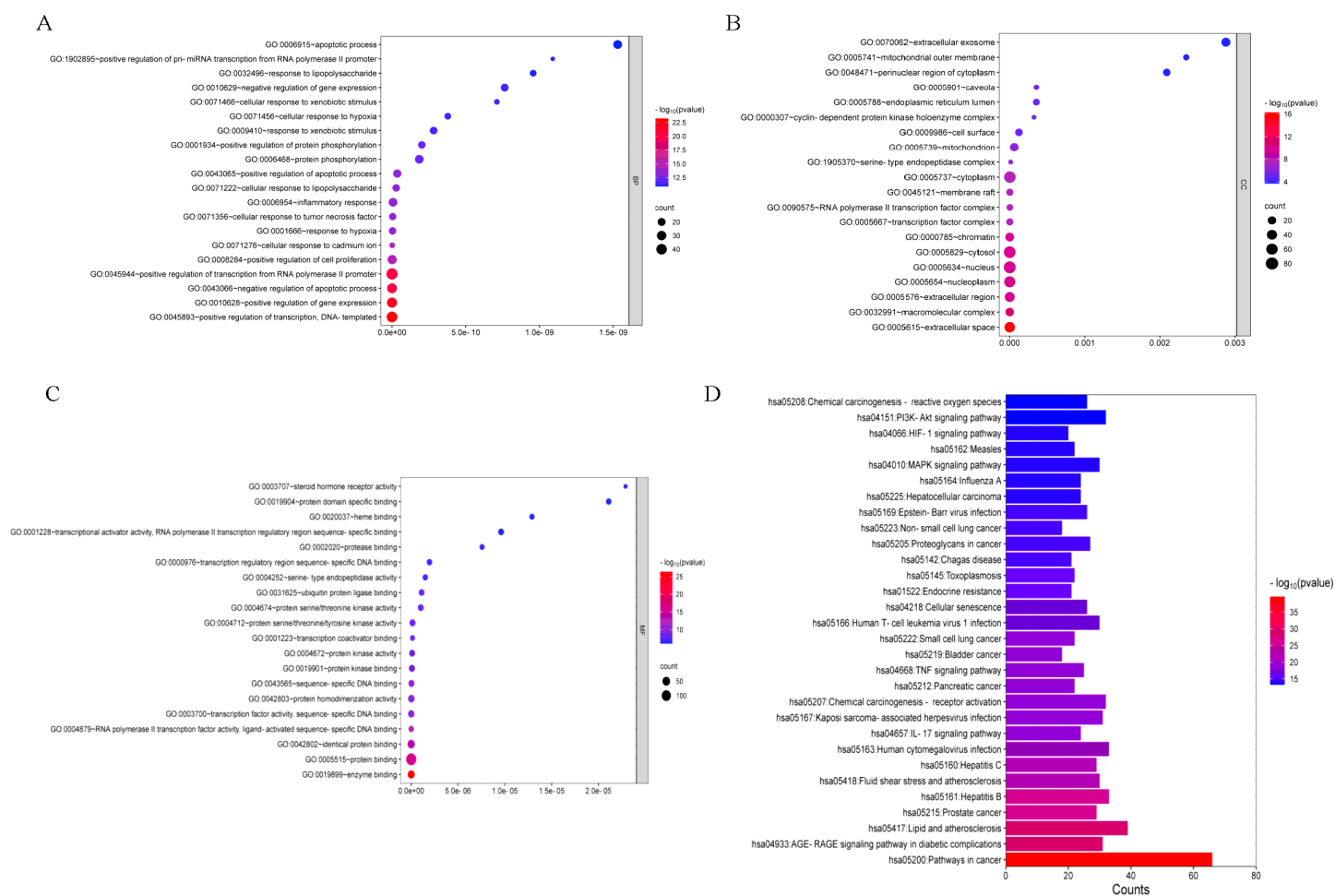


Figure 2

Bubble plot of SJL-Liver Cancer genes GO and KEGG enrichment analysis (A) The BP results showed that the targets of SJL in the treatment of liver cancer were mainly enriched in biological processes such as positive regulation of transcription, DNA template. (B) The CC analysis showed that the targets of SJL in the treatment of liver cancer were mainly enriched in extracellular spaces, macromolecular complexes, extracellular components. (C) The MF analysis showed that the targets of SJL in the treatment of liver cancer were mainly enriched in molecular functions such as enzyme binding site, protein-binding reaction. The size of the dot represents the number of genes, and the color of the dot represents the size of the p-value. (D) The abscissa represents the number of target genes in each pathway, and the ordinate represents each pathway. The pathways with higher gene numbers in the figure include pathways in cancer, PI3K, AGE-RAGE, and MAPK.

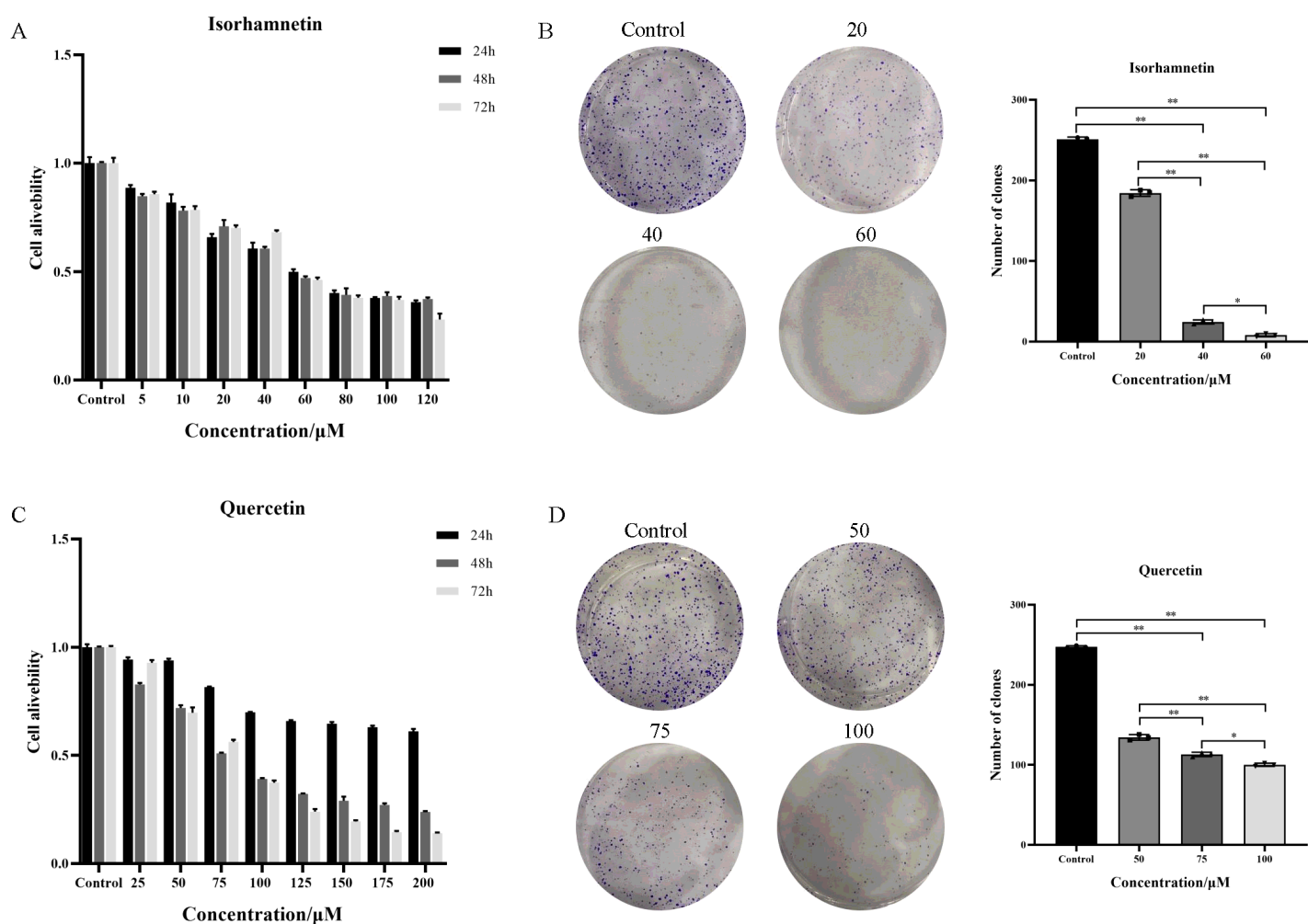


Figure 4

SJL candidate compounds inhibit the proliferation of liver cancer cells HepG2. (A) The growth curve of different concentrations of isorhamnetin treated with HepG2 cells for 24, 48, 72 h. (B) Isorhamnetin inhibited the colony formation of HepG2 cells. (C) The growth curve of different concentrations of quercetin treated with HepG2 cells for 24, 48, 72 h. (D) Quercetin inhibited the colony formation of HepG2 cells. Data were presented as mean $\pm$ SD (n=3). **p<0.01, *p<0.05.

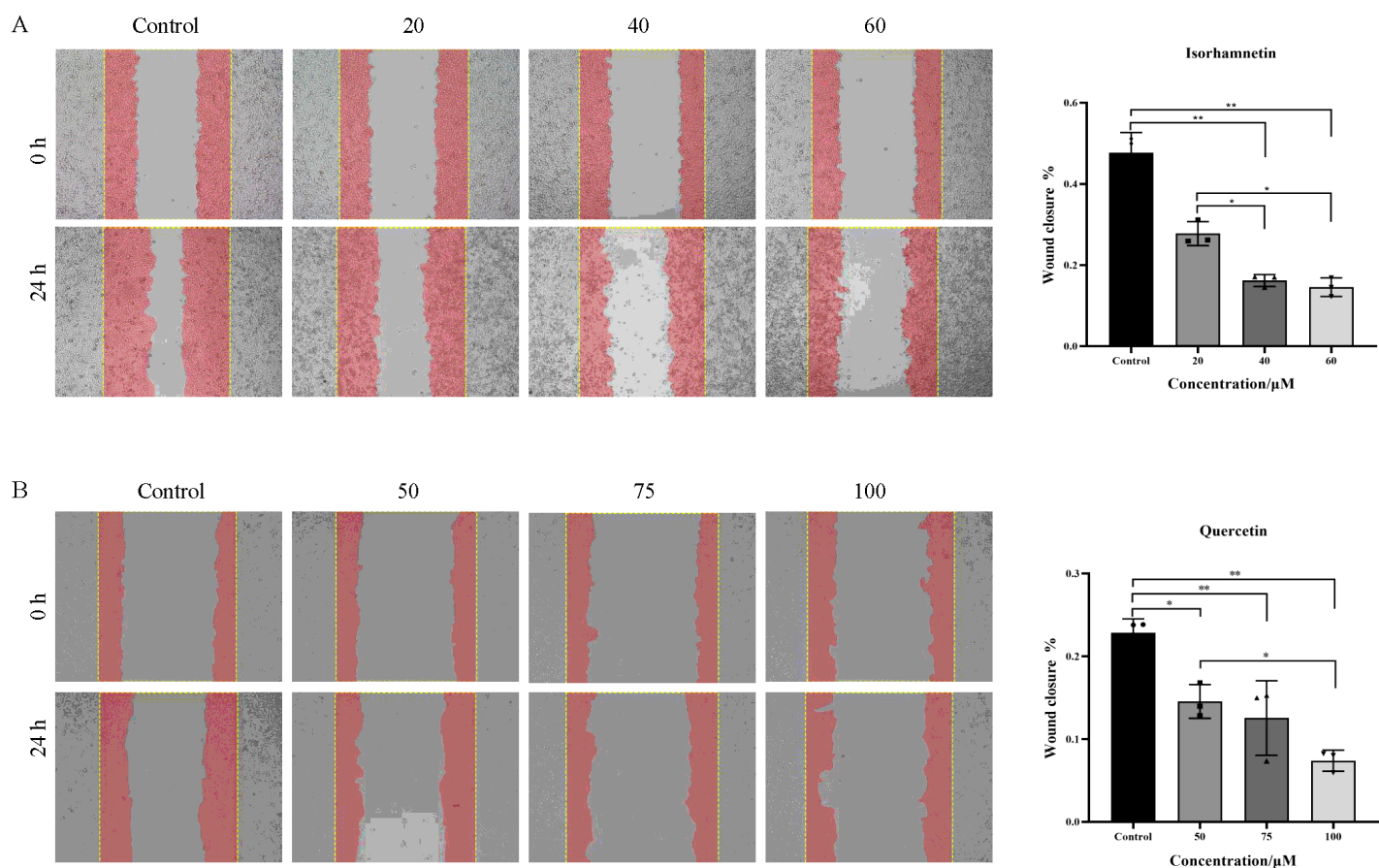


Figure 5

SJL candidate compounds inhibit the migration ability of HepG2 cells. (A) Different concentrations of isorhamnetin inhibited the migration of HepG2 cells, as determined by scratch assay. (B) Different concentrations of quercetin inhibited the migration of HepG2 cells, as determined by scratch assay. Data were presented as mean±SD (n=3). **p<0.01, *p<0.05.

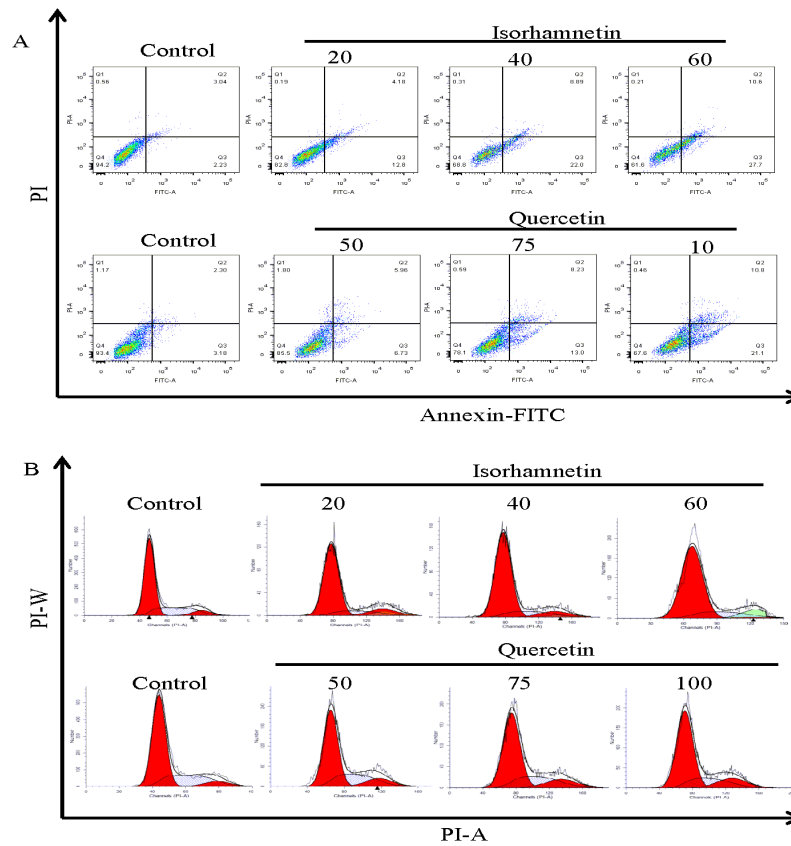


Figure 6

The effect of active ingredients from SJL on apoptosis and cell cycle of HepG2 cells. (A) Different concentrations of isorhamnetin and quercetin increased apoptosis rate in HepG2 cells. The Q2 and Q3 quadrants represent apoptotic cells. (B) Different concentrations of isorhamnetin and quercetin induced G1 blockade of HepG2 cells. The percentage of cells in the G1, S, and G2/M phases of the cell cycle was shown. Data were presented as mean±SD (n=3). **p<0.01, *p<0.05.

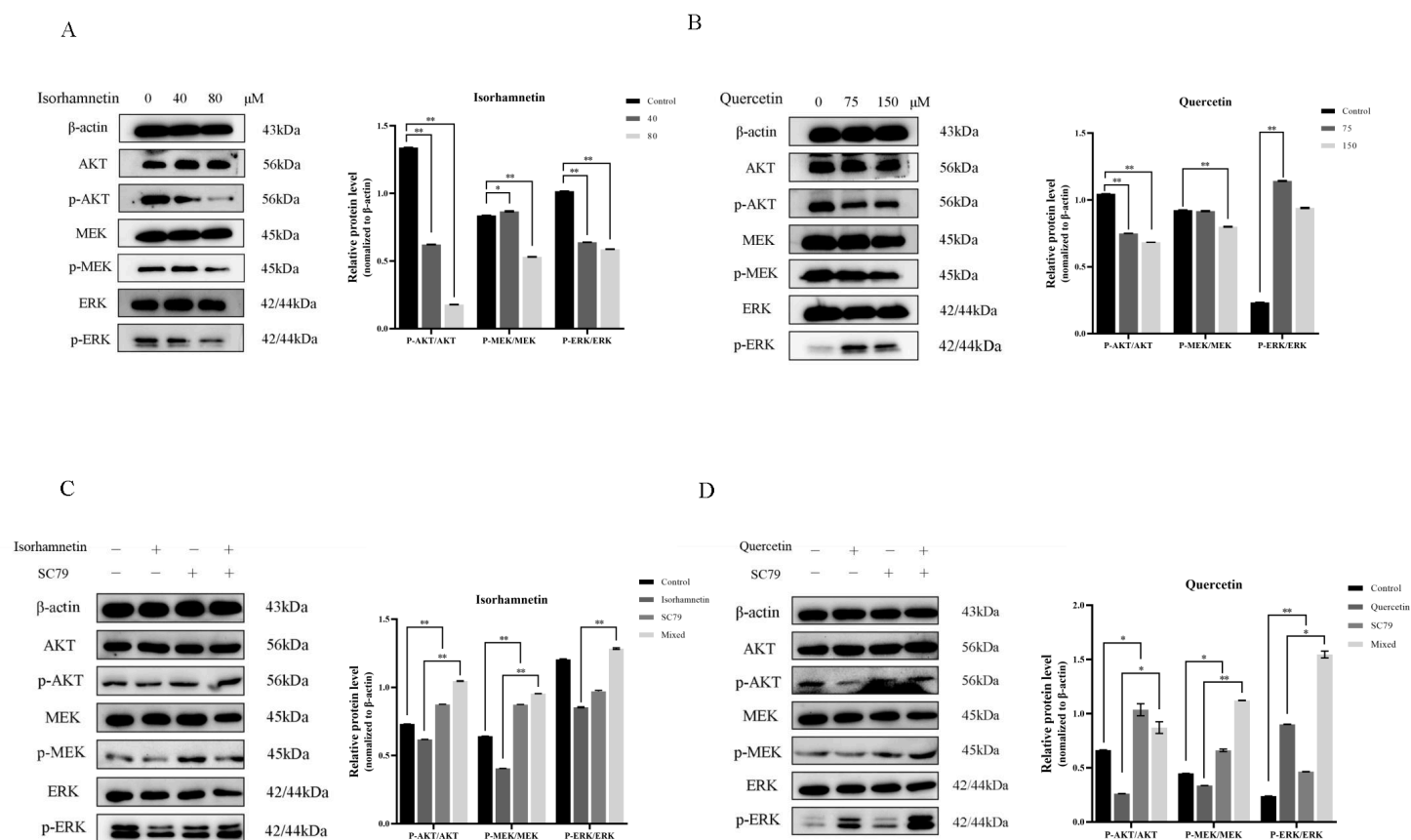


Figure 7

SJL candidate compounds induced apoptosis of hepatoma cells via the PI3K/AKT/MAPK pathway. (A) Western blot analysis of the levels of the PI3K/AKT/MAPK pathway proteins AKT, p-AKT, MEK1, p-MEK1, ERK1/2 and p-ERK1/2 induced by isorhamnetin. (B) Western blot analysis of the expressions of the PI3K/AKT/MAPK pathway proteins AKT, p-AKT, MEK1, p-MEK1, ERK1/2 and p-ERK1/2 induced by quercetin. (C) Western blot analysis of the levels of the PI3K/AKT/MAPK pathway proteins AKT, p-AKT, MEK1, p-MEK1, ERK1/2 and p-ERK1/2 induced by isorhamnetin preincubated with SC79. (D) Western blot analysis of the levels of the PI3K/AKT/MAPK pathway proteins AKT, p-AKT, MEK1, p-MEK1, ERK1/2 and p-ERK1/2 induced by quercetin preincubated with SC79. Data were presented as mean $\pm$ SD (n=3). **p<0.01, *p<0.05.

Supplementary Files

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