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Research Progress on the Anti-Liver Cancer Mechanism and Toxicity of Rhubarb Anthraquinone

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Ethnopharmacological Relevance: Rhubarb has the effect of breaking blood stasis and abnormal mass, and was often used to treat various tumor diseases including liver cancer in ancient China. Recipes containing rhubarb have anti-liver cancer properties and are still used today. However, the main components and mechanism of action of rhubarb against liver cancer are still unclear.

Aim of the Review: To conduct a review of the anti-liver cancer effects and toxicity of rhubarb anthraquinones (AQs).

Materials and Methods: This article reviewed the effects of rhubarb AQs in the treatment of liver cancer and the signaling pathways involved, and discussed the toxicity and pharmacokinetics of rhubarb AQs by searching the Web of Science, PubMed and CNKI databases.

Results: Rhubarb (Rhei Radix et Rhizoma) is a traditional Chinese medicine that has been existed for thousands of years and is used as an anti-cancer drug. Modern pharmacological research shows that rhubarb AQs, as the main component of rhubarb, contains emodin, rhein, chrysophanol, physcione and aloe-emodin, which has anti-liver cancer effects and can be considered as a potential therapeutic drug for liver cancer. However, many modern studies have shown that rhubarb AQs have certain toxicity, which hinders in-depth research on rhubarb AQs.

Conclusion: Rhubarb AQs can be used as a potential anti-liver cancer drug, but its research still has many limitations. Strengthening research on related experiments and finding a balance between toxicity and efficacy are all directions worth studying in the future.

Keywords: rhubarb anthraquinone, liver cancer, toxicity, pharmacokinetics

Introduction

Liver cancer is one of the major malignant tumors, ranking 4th and 6th in terms of global mortality and morbidity, respectively.[1] And its morbidity and mortality rates continue to increase worldwide.[2] A prediction of primary liver cancer shows that the number of new liver cancer cases per year will increase by 55.0% from 2020 to 2040.[3] The pathogenesis of liver cancer spans multiple disciplines, and research shows that liver cancer is a harmful complication of chronic viral hepatitis and alcoholic or non-alcoholic fatty liver disease (NAFLD).[4] Liver cancer has a complex pathogenesis, and liver cancer is usually diagnosed at an advanced stage, making liver cancer one of the most difficult cancers to treat. Surgery, local destructive therapy and liver transplantation are the most effective treatments for patients with early-stage liver cancer.[5] Anti-tumor therapy includes molecularly targeted drug therapy, immune checkpoint inhibitor therapy, chemotherapy and so on. First-line regimens for systemic antitumor therapy include sorafenib, tislelizumab monoclonal antibody, lenvatinib, and donafenib, and combinations include anti-PD1/PD-L1 combined with intravenous anti-VEGF drugs (atezolizumab combined with bevacizumab).[6] Second-line systemic anti-tumor therapies include regorafenib, apatinib, and ramucirumab.[7] In addition, other second-line systemic anti-tumor treatment options include FDA approval of PD-1 in combination with CTLA4 inhibitors (nivolumab in combination with ipilimumab) for patients with hepatocellular carcinoma who have progressed after prior sorafenib treatment or who cannot tolerate sorafenib.[8] However, the

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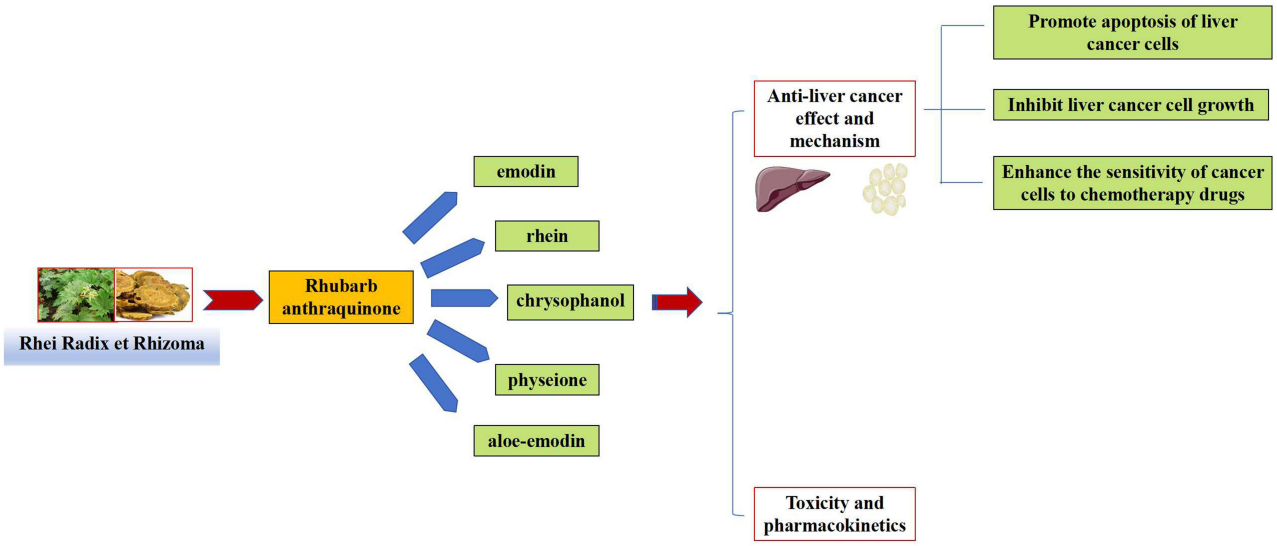
Graphical Abstract



adverse effects of antitumor therapy are numerous and are dominated by nausea, hypertension, diarrhea, malaise, and rash.[9,10] With the advancement of technology, the treatment of liver cancer is also advancing. Liver cancer genetic screening screens and identifies genes related to liver tumorigenesis and development in a high-throughput manner, which is used to explore new options for individualized targeted therapies for patients. Gene screening is performed by (1) transposon-based mutagenesis screening, (2) knockdown screening using RNA interference (RNAi) or CRISPR/Cas9 system, and (3) overexpression screening using CRISPR activation (CRISPRa) or cDNA.[11] These approaches can elucidate potential mechanisms of drug resistance and identify new HCC treatments.

In drug development, Natural products are a rich drug resource library and have become the main target for the development of anti-cancer drugs. Currently, more than 60% of clinical anti-cancer drugs are either natural products themselves or inspired by natural products.[12] For example: Icaritin soft capsule has become the first natural product drug in the world to obtain indications for liver cancer. Its combination with sorafenib can enhance the effect of inducing apoptosis in liver cancer cells.[13] Its anti-liver cancer mechanism is to increase the number and activity of cytotoxic T-cells, thus inhibiting tumor progression.[14] Therefore, the research and development of natural products has become one of the important means to find anti-liver cancer drugs. So far, a series of natural products are being clinically evaluated against cancer, such as polyphenols, Icariin, resveratrol, silybin, saponins (ginsenoside Rg3 and glycyrrhetinic acid), berberine, and the inorganic compound arsenic trioxide. Their anti-cancer mechanisms include anti-apoptosis, anti-immunity, autophagy regulation, proliferation, metastasis, angiogenesis, anti-inflammation, oxidative stress, lipid disorders, viral infections, CSC differentiation, etc.[15]

Liver cancer can be classified into categories such as “liver accumulation”, “abdominal mass” and “liver cancer” in traditional Chinese medicine. The formation of abdominal mass is mostly caused by weakness of healthy qi, disharmony of the organs, qi blockage, internal stasis of blood. Symptoms are qi stagnation, blood stasis, phlegm-dampness, and poisonous heat. Liver accumulation is caused by stasis of the liver meridians due to various reasons, the liver loses its tenderness, and fails to relieve its function. The main manifestations are pain in the right hypochondriac, or a mass under the hypochondriac, abdominal distension, lack of appetite, and liver stasis. Rhubarb (Rhei Radix et Rhizoma), included in the 2020 edition of the Chinese Pharmacopoeia, has been used as a medicine in China for thousands of years. “Shen Nong’s Materia Medica” states that rhubarb enters the stomach, large intestine, heart, and liver meridians, and tastes bitter and cold. It is mainly responsible for breaking blood stasis and abnormal mass. Zhang Xichun said in “Yixue

Zhongzhongcanxilu": Rhubarb is bitter in taste, fragrant and cool in nature. It can enter the bloodstream and eliminate all blood stasis. Because of its fragrant aroma, it can also be used to regulate qi and treat qi stagnation and pain when used sparingly. Its power sinks but does not float. It mainly plays the role of offense and decision-making, and eliminate all the abdominal mass. Therefore, in ancient China, rhubarb was often used to treat various tumor diseases, including liver cancer. "The Synopsis of the Golden Chamber" records a prescription called Dahuang Qichong pills, which has been used for thousands of years as a common anti-liver cancer prescription. The prescription uses rhubarb as the main medicine, and its function of passing through the twelve meridians, promoting blood circulation and removing blood stasis. The whole prescription has the effects of promoting blood circulation and breaking blood stasis, dredging menstruation and eliminating abnormal mass, and is used for abnormal mass caused by internal stasis of blood. Symptoms include abdominal mass, disordered skin and nails, dark complexion, hot flashes and weight loss. Moreover, Dahuang Qichong pills have been used to this day and are now sold in the Chinese market as a proprietary Chinese medicine for the treatment of liver cancer. With the development of scientific research on modern drugs, the pharmacological effects of rhubarb on liver cancer have been widely studied. Modern research believes that rhubarb mainly contains flavonoids, saponins, AQs and other compounds.[16] After reviewing the literature, there are no reports on the anti-liver cancer effects of rhubarb flavonoids and saponins, but there are many reports on the anti-liver cancer effects of rhubarb AQs. Therefore, it is speculated that AQs should be the main anti-liver cancer substances of rhubarb.

AQs are a class of natural quinone compounds that are widely found in natural plants. Modern research shows that AQ compounds have various pharmacological effects, such as anti-inflammatory, antibacterial, antioxidant, lipid-lowering, and anti-tumor etc.[17–21] In recent years, the anti-cancer effects of AQ compounds have been widely studied and are considered to be potential therapeutic drugs for lung cancer, colon cancer, liver cancer, prostate cancer and other cancers. Drugs derived from AQ compounds, such as doxorubicin (DOX), Mitoxantrone, epirubicin, idarubicin, and varubicin have been successfully used in the chemotherapy of malignant tumors and solid tumors.[22] Rhubarb AQs are mainly including emodin, rhein, chrysophanol, aloe-emodin and physcion and corresponding glycosides.[23] Many in vivo and in vitro experiments have confirmed that rhubarb AQs can inhibit the growth of liver cancer cells. However, modern studies have shown that rhubarb AQs have certain toxicity, which has hindered in-depth research on rhubarb AQs. In view of the fact that there is still a lack of review on the anti-liver cancer effects and toxicity of rhubarb AQs. Therefore, this article conducts a review of the treatment of liver cancer by rhubarb AQs and the signaling pathways involved by searching the Web of Science, PubMed and CNKI databases before 2024, enter keywords such as rhubarb / anthraquinone /emodin/rhein/chrysophanol/physcion/aloe-emodin / anti-hepatic cancer / toxicity / pharmacokinetics, and conducts a study on the toxicity and pharmacokinetics of rhubarb AQs. This study aims to provide guidance and basis for the research on the anti-liver cancer of rhubarb AQs.

Main Components and Molecular Structure of Rhubarb AQs

The main components of rhubarb AQs include emodin, rhein, chrysophanol, physcion, and aloe-emodin and corresponding glycoside. These ingredients are the main quality testing indicators of the 2020 version of "Chinese Pharmacopoeia", and are also widely found in Chinese medicinal materials such as Polygonum multiflorum Thunb, and Polygonum cuspidatum Sieb. and Zucc. The emodin, rhein, chrysophanol, physcion, and aloe-emodin in this review are yellow crystals, with needle -shaped and chip -shaped, molecular weight, density, and more physical and chemical properties as shown in Table 1 (data from www.chemicalbook.com /). Their chemical structures are shown in Figure 1.

Pharmacological Effects and Mechanisms of Rhubarb AQs Against Liver Cancer

Rhubarb extract has been confirmed to have anti-liver cancer effects. Kun-Hsi Tsai et al extracted rhubarb with water and found that rhubarb extract inhibited the migration ability of HA22T cells in wound healing, migration and invasion assays in a dose-dependent manner.[24] Another study showed that methanol extract of rhubarb can significantly reduce the levels of tumor markers alpha fetoprotein (AFP) and glutamyl transpeptidase(GGT) in hepatocellular carcinoma cell lines (HCC) of rats, which has obvious anti-liver cancer effects. In addition, Liquid chromatography-tandem mass spectrometry (LC-MS)/MS analysis found that the methanol extract of rhubarb mainly contained 16 components, including AQ, flavonoids and tannin

Table 1 The Physicochemical Properties of Emodin, Rhein, Chrysophanol, Physcion, and Aloe-Emodin

Name	Emodin	Rhein	Chrysophanol	Physcione	Aloe emodin
Alias	6-Methyl-1,3,8-trihydroxyanthraquinone	4,5-dihydroxy-9,10-dioxoanthracene-2-carboxylic acid	1,8-dihydroxy-3-methylanthracene-9,10-dione	1,8-dihydroxy-3-methoxy-6-methylanthracene-9,10-dione	1,8-dihydroxy-3-(hydroxymethyl) anthracene-9,10-dione
Source	Rhubarb	Rhubarb	Rhubarb	Rhubarb	Rhubarb
CAS number	518–82-1	478–43-3	481–74-3	521–61-9	481–72-1
EINECS number	208–258-8	207–521-4	207–572-2	208–315-7	207–571-7
Compound type	Anthraquinone	Anthraquinone	Anthraquinone	Anthraquinone	Anthraquinone
Molecular formula	C15H10O5	C15H8O6	C15H10O4	C16H12O5	C15H10O5
Molecular weight	270.24	284.22	254.24	284.26	270.24
Properties	Powder	Needles or powder	Crystalline solid or powder	Crystalline solid	Powder
Color	Orange	Yellow-brown	Yellow or brown	Yellow to dark yellow to dark orange	Brown to orange
Solubility	<0.1 g/100 mL at 19 °C	<0.1 g/100 mL at 17 °C	<0.1 g/100 mL at 18 °C	Almost insoluble in water	Slightly dissolved in trichloromethane
Density	1.3280 g/cm3	1.7±0.1 g/cm3	1.5±0.1 g/cm3	1.4±0.1 g/cm3	1.6±0.1 g/cm3
pKa	6.39±0.20(Predicted)	3.17±0.20(Predicted)	6.63±0.20(Predicted)	6.23±0.20(Predicted)	6.30±0.20(Predicted)
Boiling point	373.35°C at 760 mmHg	597.8±50.0 °C at 760 mmHg	489.5±45.0 °C at 760 mmHg	560.5±50.0 °C at 760 mmHg	568.8±50.0 °C at 760 mmHg
Flash point	322.8±23.6 °C	329.4±26.6 °C	263.9±25.2 °C	215.4±23.6 °C	311.9±26.6 °C
Vapour pressure	0.0±1.7 mmHg at 25°C	0.0±1.8 mmHg at 25°C	0.0±1.3 mmHg at 25°C	0.0±1.6 mmHg at 25°C	0.0±1.6 mmHg at 25°C
Refractivity	1.745	1.761	1.71	1.678	1.746
Polar surface area	94.83	112 Å 2	75Å 2	84Å 2	95Å 2
LogP	3.641 (est)	4.58	5.03	5.2	3.38
Storage conditions	2–8°C,Cool, dry and sealed	2–8°C	Inert atmosphere,Room Temperature	−20°C Freezer, Under Inert Atmosphere	2–8°C

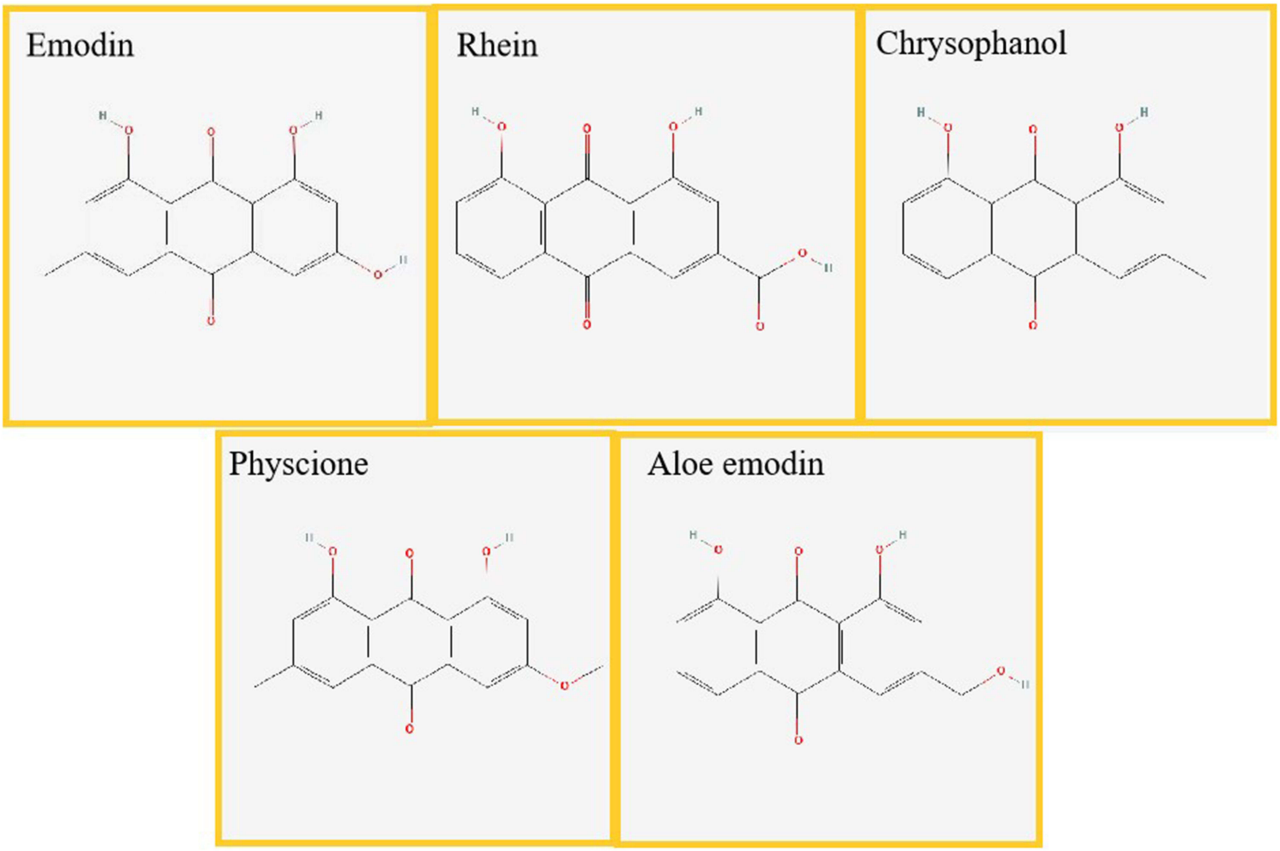


Figure 1 The chemical structure of emodin, rhein, chrysophanol, physcion, and aloe-emodin.

compounds, and a total of 7 AQs were detected.[25] The AQs of rhubarb include AQ glycosides and AQ aglycones. AQ glycosides are the components of rhubarb that stimulate the intestines and cause diarrhea, while AQ aglycones have anti-tumor effects.[26] During the literature search, we also found that the anti-cancer component of rhubarb is mainly AQ aglycone. Therefore, this review selected AQ aglycones with antitumor effects in rhubarb for study. It was found that there are currently many literatures reporting the anti-liver cancer effects of emodin, rhein, chrysophanol, physcion and aloe-emodin among rhubarb AQs. Therefore, this review mainly focuses on the anti-liver cancer effects of the above-mentioned compounds. The anti-liver cancer efficacy of rhubarb AQs are shown in Table 2.

The Anti-Liver Cancer Effect and Mechanism of Emodin

Emodin(6-Methyl-1,3,8-trihydroxyanthraquinone) is a naturally occurring AQ compound that has anti-inflammatory, antioxidant, hepatoprotective, and anti-cancer pharmacological effects.[55–57] With the deepening of research on emodin, emodin has also been confirmed to have a variety of hepatoprotective effects, including NAFLD, liver fibrosis, and liver cancer.[58] Liver cancer will occur with the progression of NAFLD and liver fibrosis. Therefore, emodin may be used as an early interference drug for liver disease to prevent the occurrence of liver cancer. Based on current research, the anti-liver cancer effect of emodin is mainly related to promoting the apoptosis of liver cancer cells, inhibiting the growth of liver cancer cells and regulating the energy metabolism of liver cancer cells. In terms of promoting apoptosis of liver cancer cells, research by Jian-Qing Yu et al found that emodin (30, 60, 90, 120 μM) can induce apoptosis of human hepatoma G2 (HepG2) cells and make HepG2 cells stay in the G1 phase. Its molecular mechanism involves the increase of P53 protein and the decrease of nuclear factor kappa B/p65 (NF-kB/p65) protein. In addition, emodin can activate the expression of cysteinyl aspartate specific proteinase-8 (caspase-8) and caspase-9 in the Caspases family involved in inducing apoptosis, which indicates that emodin can promote apoptosis of HepG2 cells.[27] Among them, P53 can promote apoptosis through transcription-dependent and -independent mechanisms.[59] NF-κB is also involved in the apoptotic pathway.[60] NF-κB has been increasingly used in cancer research in recent years,[61,62] its

Table 2 The Anti-Liver Cancer Efficacy of Emodin, Rhein, Chrysophanol, Physcion, and Aloe-Emodin

Pharmacological Effects	Models	Dosing Method	Dosage of Administration	Molecular Mechanisms	References
Emodin	HepG2 cells	Incubate	30, 60, 90, 120μM	Promote HepG2 cells apoptosis	[27]
	Male BALB/c nude mice	i.h	1 mg/kg, 10 mg/kg	Inhibiting the VEGFR2-AKT-ERK1/2signaling pathway and promoting a miR-34a-mediated signaling pathway	[28]
	SMMC-7721 cells	Incubate	100 μmol/l	Relate to the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/AKT signaling pathways	[29]
	HCC Mahlavu, PLC/PRF/5 and HepG2	Incubate	2.5 μg/mL	Activation of caspase-9 and caspase-3 leads to apoptosis	[30]
	HCC	Incubate	20 μM	Inhibits the activity of SREBP-2, thereby inhibiting AKT signaling and inactivating STAT3	[31]
	Bel-7402 cells	Incubate	100 mmol/L	Induces apoptosis by inhibiting SREBP-1	[32]
	Liver cancer cell lines	Incubate	40 μM, 80 μM	Mediated by cellular autophagy through the miR-371a-5p/PTEN axis	[33]
	HepG2 cells	Incubate	60 μM	Inhibits PI3K/AKT/mTOR and Wnt/β-catenin pathways	[34]
	HepG2 cells	Incubate	40, 25 and 16 μM	Inhibited glycolysis	[35]
	HepG2 cells	Incubate	50mM	Impair mitochondrial function	[36]
	HepG2 cells	Incubate	40 mM	Induction of apoptosis in HepG2 cells via mitochondrial pathway and/or death receptor pathway	[37]
	HCC	Incubate	50 mg/kg	Regulates M2 polarization via miR-26a/TGF-β1/Akt	[38]
Rhein	HepG2 and Huh7 cells	Incubate	50, 100, 150 and 200 μmol/L	Induces ROS production and activates JNK/Jun/caspase-3 signaling pathway	[39]
	SMMC-7721/DOX cells	Incubate	20 μM,40 μM,60 μM,80 μM	Inhibits energy metabolism and induces mPTP opening	[40]
	HepG2 cells	Incubate		Inhibit the expression of HSP72, HSC70 and GRP78	[41]
	HepG2 cells	Incubate	12 μM,24 μM,36.3 μM,48 μM	Activation of Casp-3 and −9 genes and DNA fragmentation.	[42]
	HepG2 cells	Incubate	35 μM, 70 μM	Apoptotic system involving p53 and CD95/CD95L	[43]
	HepG2 cells	Incubate	75 μM,100 μM	Activation of Fas and mitochondria-mediated apoptotic pathways	[44]
Chrysophanol	J5 human liver cancer cells	Incubate	120 μM	Induces ROS production, DNA damage, mitochondrial dysfunction, ATP loss, and enhanced LDH activity	[45]
	Hep3B cells	Incubate	50 μM	Decreases mitochondrial membrane potential ($\Delta\Psi m$) and ATP levels	[46]
	HepG2 cells	Incubate	50 μmol/L, 100 μmol/L	Affects mitochondrial function	[47]
Physcion	HCC cell	Incubate	5 μM,10 μM	Induces apoptosis via mitochondrial pathway by targeting miR-370	[48]
	Huh7-SR and HepG2-SR cells	Incubate	20 μM	Regulation of the miR-370/PIM1 axis	[49]
Aloe emodin	HepG2 and Hep 3B cells	Incubate	10 μg/mL,20 μg/mL	Increase the expression of p5 protein, thereby stimulating the expression of its downstream p21 and Bax proteins	[50]
	HepG2 cells	Incubate	40 μM	Enhances expression of proteins involves oxidative stress and cell cycle arrest	[51]
	HepG2 cells	Incubate	50 μM, 100 μM	Regulate PI3K-AKT signaling pathway	[52]
	HepG2 cells	Incubate	40 μM	Activate JNK signaling pathway	[53]
	Hep3B cells	Incubate	60 μM	Regulate P53 signaling mechanism	[54]

involvement in inflammation and immune response has been clearly studied, but its role in cross-regulation with P53 in promoting apoptosis of liver cancer cells still deserves further study.[63] In addition, Xubin Jing et al added emodin (2.5 μg/mL) to human HCC and HepG2 cells, and found that emodin can affect intracellular metabolism by generating Reactive oxygen species (ROS) in these cells, which affects the transmembrane potential of mitochondria and activates caspase-9 and caspase-3, leading to DNA fragmentation and promoting cell apoptosis.[30] In addition, emodin (40 mM) can also improve the Bcl-2-associated X protein/B-cell lymphoma/lerkemia-2 (Bax/Bcl-2) ratio in HepG2 cells, cause damage to mitochondrial membrane potential (MMP), and promote HepG2 cell apoptosis. It can also activate Fatty acid synthase (Fas), Fas ligand (Fas-L), caspase-8, truncated BID (tBid). and caspase-3, thereby stimulating death receptor apoptotic signaling. On the other hand, emodin can effectively attenuate the phosphorylation of protein Kinase B(Akt) and extracellular signal-regulated protein kinase (ERK) and promote the phosphorylation of p38, thereby regulating apoptosis mediated by the mitochondrial pathway and/or the death receptor pathway. However, emodin had no toxic effect on primary human hepatocytes.[37] Excessive activation of the phosphatidylinositol-3-kinase/AKT (PI3K/AKT) signaling pathway is closely related to cancer.[64] Emodin-mediated PI3K/AKT signaling seems to play an important role in inhibiting the growth of liver cancer cells. The PI3K signaling cascade is closely related to cell proliferation, survival, growth, movement and activation, and plays a key role in the development and progression of cancer.[65] Qin B's research found that emodin can induce S phase and G2/M phase arrest of HepG2 cells, and promote HepG2 cell apoptosis. Differential expression gene analysis shows that the anti-liver cancer mechanism is related to cell adhesion, cancer metastasis and cell cycle arrest. The molecular level involved is mainly the inhibition of PI3K/AKT/Mammalian target of rapamycin (PI3K/AKT/mTOR)-induced autophagy in liver cancer cells and the wingless/β-catenin(Wnt/β-catenin) pathway inducing tumor epithelial-mesenchymal transition (EMT). This study also showed that emodin (60 μM) down-regulated the phosphorylation of glycogen Synthase Kinase 3β (GSK3β) in HepG2 cells, and GSK3β, as a key downstream regulator of the Wnt pathway and a direct target of AKT, has been shown to induce autophagy.[34,66] In addition, emodin (40 μM, 80 μM) can also regulate autophagy and inhibit the growth of liver malignant tumors by regulating the miR-371a-5p axis and acting on phosphatase and tensin homolog (PTEN).[33] The body can maintain cell homeostasis through autophagy, thereby intervening in the occurrence of cancer. Anticancer drugs commonly used in clinical practice, such as rapamycin and chloroquine, are autophagy regulators.[67] In addition, emodin can also inhibit the proliferation of SMMC-7721 cells in a concentration- and time-dependent manner. This may be related to emodin's ability to regulate mitogen-activated protein kinase (MAPK) and PI3K/AKT signaling pathways.[29] Moreover, emodin can inhibit the expression of P-AKT.[68] Clinical studies have found that p-AKT is highly expressed in tumor tissue but absent in normal liver.[69] Therefore, P-AKT may be a potential target for emodin in the treatment of liver cancer. The regulation of MAPK signaling pathway by emodin includes promoting the phosphorylation of ERK and p38, mildly inhibiting the expression of phosphorylated c-Jun N-terminal kinase (p-JNK).[29] It is well known that the MAPK pathway is closely related to tumor cell proliferation, apoptosis, invasion and tumor metastasis.[70] MAPKs include ERK, P38 and JNK, and inhibition of ERK and p38 phosphorylation induces apoptosis and cycle arrest in HCC cells.[71] However, some studies have confirmed that activation of the p38/ERK MAPK pathway will induce apoptosis and S-phase arrest in HCC cells (increased phosphorylation of p38 and ERK1/2).[72] Many studies have shown that JNK is continuously activated in most HCC cells, but its exact role in carcinogenesis remains controversial. There are also research results showing that JNK inhibition increases the sensitivity of liver cancer cells to apoptosis.[73] Therefore, the anti-liver cancer mechanism in which MAPK participates deserves further study. Mitochondria are the energy factories of cells, providing basic materials for tumorigenesis and metabolism, participating in transcriptional regulation, and controlling the death of cancer cells.[74] Emodin can promote apoptosis by inducing mitochondrial dysfunction in liver cancer cells, this is related to the mitochondrial matrix-specific protein cyclophilin D (CypD) inducing mitochondrial permeablity transition pore (mPTP) opening and leading to mitochondria-mediated cell death. Studies have found that emodin (50mM) induces mitochondrial dysfunction in HepG2 cells by enriching mitochondria, which is reflected in reducing the production of MMP and adenosine triphosphate (ATP) in a dose- and time-dependent manner, thereby inducing HepG2 cells apoptosis.[36] The growth of liver tumors requires adequate blood supply. The kinase vascular endothelial growth factor receptor 2 (VEGFR2) plays a key role in the angiogenesis process.[75] Emodin can inhibit the activity of VEGFR2, Jianguo Bai et al injected emodin (1 mg/kg, 10 mg/kg) intravenously into nude mice injected with HepG2 cells, the results showed that emodin can inhibit tumor volume and tumor weight, which is related to the inhibition of the VEGFR2-AKT-ERK1/2 signaling pathway, and is also related to the reduction of tumor suppressor mothers against decapentaplegic homolog 4 (SMAD4) and p-SMAD2 protein levels by promoting the expression of miR-34a protein.[28]

Liver cancer cells mainly obtain energy through aerobic glycolysis.[76] Studies have found that emodin (40, 25 and 16 μM) can down-regulate the expression of genes related to glycolysis (such as hexokinase II (HKII), pyruvate kinase isozyme typeM2 (PKM2) and lactate dehydrogenase (LDHA)) and trigger the production of intracellular ROS, leading to increased mitochondrial damage and apoptosis.[35] However, since liver cancer cells are often in a microenvironment lacking oxygen and sugar, one of the main pathways of their metabolism is lipid metabolism.[76] Rhubarb can participate in the survival and proliferation of liver cancer cells by affecting the lipid metabolism process. A study confirmed that emodin (100 mmol/L) can inhibit the expression of sterol regulatory element binding protein 1 (SREBP1) and its downstream genes ATP citrate lyase (ACLY), acetyl-CoA carboxylases alpha (ACACA) and FASN in HCC cells, thus affecting the lipid metabolism and induction of apoptosis.[32] In addition, the combination of emodin and other anti-tumor drugs will also improve the anti-tumor effect. Kim Y S et al combined emodin (20 μM) and sorafenib and found that emodin enhances the inhibitory effect of sorafenib in HCC cells in a time-dependent manner, and it can reduce the resistance of liver cancer cells to sorafenib and synergistically increase the stasis phase of HCC cells in the G1 phase. Its mechanism is related to emodin inhibiting the activity of SREBP-2, thereby inhibiting oncogenic AKT signaling and inactivating the oncogenic transcription factor signal transducers and activators of transcription 3 (STAT3).[31] The anti-liver cancer effect of new preparations made by combining emodin with other drugs is also vary significant. For example, Li C's research explored a new pharmaceutical compound β-dihydroartemisinin-emodin (β-DHA -emodin), which can induce HepG-2 cell apoptosis by up-regulating the expression of caspase-3/8/9 and Bax and down-regulating the expression of Bcl-2.[77] In the process of treating liver cancer, cisplatin will reduce the therapeutic effect due to its drug resistance. The combination of emodin and cisplatin can enhance the sensitivity of HepG2 cells to cisplatin by inhibiting EMT, thereby playing a role in prevent recurrence and metastasis of liver cancer.[78] In addition, emodin can inhibit anthracycline reductase to prevent liver cancer cells from becoming resistant to anthracyclines, thereby enhancing the effect of anthracyclines in the treatment of liver cancer.[79] Dang Z's research also found that the combination of emodin and chloroquine will promote the apoptosis of HepG2 and Huh7 cancer cells and promote the arrest of liver cancer cells in the sub-G1 phase.[80] In addition, emodin (40 μM) can also synergize with radiation therapy to promote HepG2 cells death and prolong the G2/M phase arrest of HepG2 cells.[81] It can be seen that there are many studies on the anti-liver cancer effect of emodin, including in vivo and in vitro experiments. It is suggested that emodin may be a potential drug to be developed as an anti-liver cancer drug. The mechanism of emodin anti -liver cancer is shown in Figure 2.

The Anti-Liver Cancer Effect and Mechanism of Rhein

Rhein (4,5-dihydroxy-9,10-dioxoanthracene-2-carboxyli acid) is a lipophilic AQ compound with pharmacological effects of hepatoprotective, renal protective, anti-inflammatory, antioxidant, anticancer and antibacterial activities.[82–87] The therapeutic mechanism of rhein for liver cancer mainly includes mitochondrial damage and autophagy-mediated

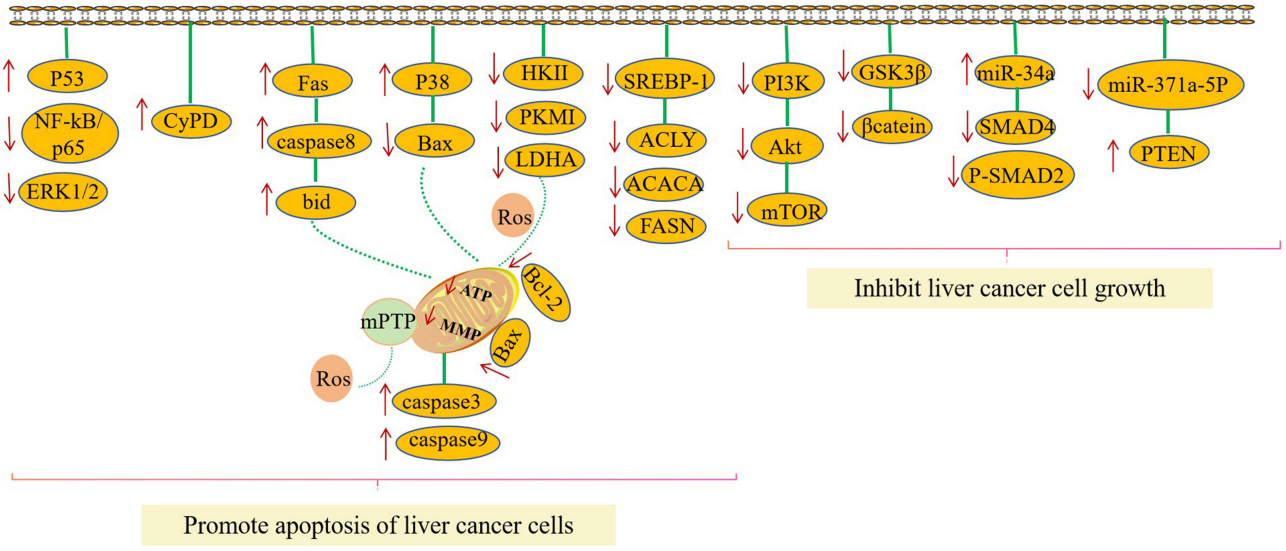


Figure 2 The molecular mechanism of emodin anti -liver cancer.

apoptosis of liver cancer cells. Rhein (75 μM, 100 μM) can increase the protein expression of Fas, p53, p21, Bax, caspase-3, −8, −9 and Poly(ADP-ribose) polymerase (PARP), and reduce the protein expression of Bcl-2, cyclin A, and cyclin-dependent kinase 2 (CDK 2) in HepG2 cells, thereby arresting HepG2 cells in the S phase and activating the mitochondria-mediated apoptotic pathway.[44] P53 is related to DNA repair, cell cycle arrest, and aging, and it can regulate cell apoptosis and participate in tumor suppression.[88] It is rapidly activated when cells encounter oxidative damage, thereby inducing the mitochondrial apoptosis pathway by regulating Bax, Bcl-2 and cleaved caspase.[89,90] The death receptor-mediated pathway and the mitochondria-mediated apoptosis pathway will activate the effector caspase and the downstream substrate PARP of caspase-3, thus directly leading to apoptosis.[91] In addition, Al-fatlawi A A's research showed that rhein (12 μM, 24 μM, 36.3 μM, 48 μM) can induce HepG2 cell apoptosis in a dependent manner by activating p53 and caspase, mainly by up-regulating p53 and Bax genes and down-regulating Bcl- 2 gene, thereby activating caspase-3 and −9 genes and DNA fragmentation.[42] In addition, aPO-1/Fas (CD95), as a downstream molecule of p53, can enhance the sensitivity of HepG2 cells to apoptosis.[92] Rhein can enhance the expression of CD95 ligands (mCD95L, sCD95L),[43] which may be the main reason for the apoptosis-inducing effect of rhein. In addition to promoting cell apoptosis by increasing the expression of P53, rhein can also promote cell cycle arrest of liver cancer cells through P53. Rhein (35 μM, 70 μM) can significantly increase the expression of p53 in HepG2 cells, thereby increasing the expression of p21/WAF1 protein, leading to HepG2 cells cycle arrest in the G1 phase.[92] In terms of promoting apoptosis of liver cancer cells, research by Wang A et al also found that rhein (50, 100, 150 and 200 μmol/L) significantly inhibited the viability of HepG2 and Huh7 cells in a dose-dependent and time-dependent manner, its mechanism of inducing apoptosis in liver cancer cells is related to promoting the production of ROS in liver cancer cells and activating the JNK/Jun/caspase-3 signaling pathway.[39] The anticancer activity of rhein is also considered to be related to inducing mPTP opening and causing mitochondrial damage in liver cancer cells.[93] The research results of Du Q et al indicated that rhein (100 μM) can directly act on the mitochondria of Hep-G2cells, causing MPT and releasing Ca2+ and Cyto c into the cytoplasm, and triggering caspase 3 activation, thereby inducing subsequent cell death process.[93] Rhein treatment can increase the sensitivity of SMMC-7721 cells to DOX and promote apoptosis. The mechanism may involve rhein inhibiting mitochondrial energy metabolism in SMMC-7721 cells, reducing cellular ATP and adenosine diphosphate (ADP) levels, and changing the relationship between ATP and ADP Ratio. These effects may be due to rhein affecting the functions of voltage dependent anion channel (VDAC) and adenine nucleotide translocase (ANT) associated with the mPTP complex and inhibiting ATP transport.[40] In addition, another study also showed that the combination of rhein and DOX has synergistic anti-liver cancer activity. The reduction of mitochondrial inner membrane potential ($\Delta\Psi m$) and the opening of mPTP inhibits the exchange of ATP/ADP between the mitochondrial matrix and cytoplasm, thereby affecting the accumulation of DOX in liver cancer cells.[94] Quality control of proteins is also an important way to combat cancer cell proliferation.[95] Modern research has confirmed that HSP72, HSC70 and GRP78 are a potential targets for cancer treatment.[96] HSP72 participates in the HepG2 cells death pathway by interacting with pro-apoptotic factors.[97] Activation of HSP70 through the mitogen-activated protein MAPK/ERK negative feedback pathway reduces oncogenic signaling and impairs HCC cells development and progression.[98] GRP78-mediated endoplasmic reticulum stress(ERS) plays an important role in the regulation of liver malignancies.[99] Research by Wang J et al found that rhein can inhibit the expression of heat Shock Protein 72 (HSP72), heat-shock cognate 70 (HSC70) and glucose-regulated protein 78 (GRP78) in HepG2 cells, thereby inhibiting the occurrence of liver cancer. Moreover, the dual inhibition of HSP72 and HSC70 by rhein can enhance the sensitivity of liver cancer cells to artemisinin derivatives. Therefore, the combination of artemisinin derivatives and rhein has significant efficacy in the treatment of liver cancer.[41] In addition, at non-cytotoxic concentrations, rhein (20μM, 30μM) can enhance the resistance to chemotherapy drugs when combined with chemotherapy drugs (docetaxel, paclitaxel and vincristine resistant). The mechanism of rhein anti -liver cancer is shown in Figure 3.

The Anti-Liver Cancer Effect and Mechanism of Chrysophanol

Chrysophanol (1,8-dihydroxy-3-methylanthracene-9,10-dione) is the most abundant free AQ compound in rhubarb and has anti-inflammatory, anti-cancer, and neuroprotective effects.[100–102] There are few studies on chrysophanol's anti-liver cancer effect, and its anti-liver cancer mechanism is mainly reflected in affecting the function of mitochondria. Chrysophanol (120 μM) induces ROS production, DNA damage, mitochondrial dysfunction, ATP loss, and increased

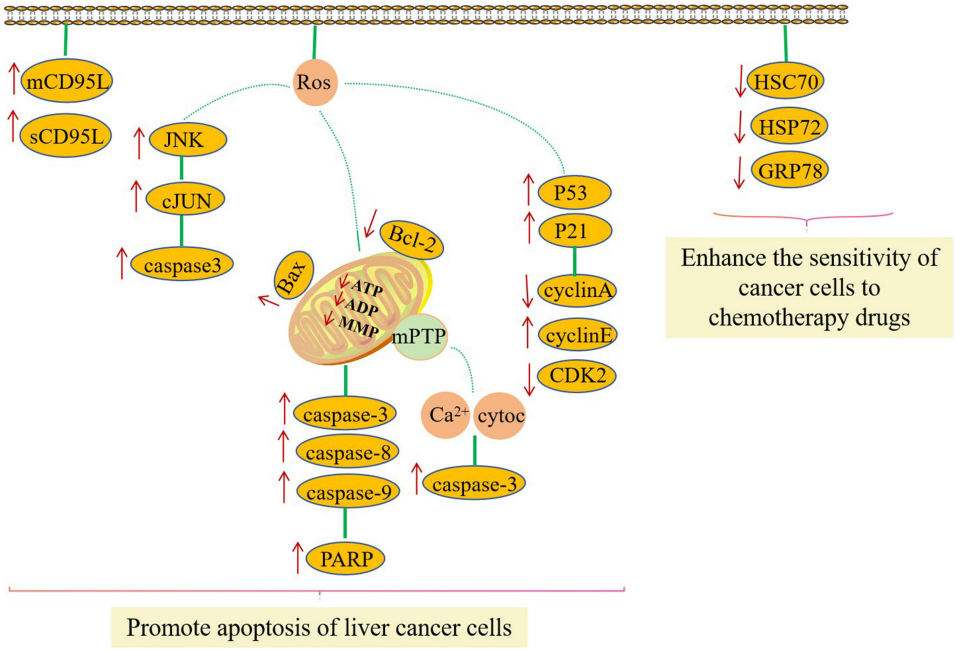


Figure 3 The molecular mechanism of rhein anti -liver cancer.

lactate dehydrogenase (LDH) activity in liver cancer cells, leading to cell necrosis. However, it has no activation effect on caspase-3, which directly causes apoptosis of cancer cells.[45] Ni CH et al found that chrysophanol (50 μM) induced the production of ROS and Ca^{2+} in Hep3B cells, and reduced ΔΨm and ATP levels, leading to necrotic cell death. In addition, chrysophanol can also increase the protein levels of p27 and p21. p21 can act as an inhibitor of CDK, reducing the expression of CDK2, cyclin D and thymidylate synthase (TSase) proteins, thereby arresting Hep3B cells in the S phase.[46] P21 has been shown to be a key mediator of cell cycle arrest in the G1 phase, preventing the transition to G1/S,[103] and p27 inhibits the activation of the cyclin E-CDK2 complex and plays a key role in the progression of the cell cycle from the G1 phase to the S phase.[104] In addition, Xie Yan's research found that chrysophanol can induce liver cancer cells death by affecting mitochondrial function, and chrysophanol (50 μmol/l, 100 μmol/l) can enhance the expression of CyPD protein, which is a molecular component and regulator of mPTP and is involved in the apoptosis process of liver cancer cells.[47] The mechanism of chrysophanol anti -liver cancer is shown in Figure 4.

The Anti-Liver Cancer Effect and Mechanism of Physcion

Physcion (1,8-dihydroxy-3-methoxy-6-methylanthracene-9,10-dione) is an AQ derivative widely isolated and characterized from terrestrial and marine sources and has laxative, antitumor, anti-inflammatory, antibacterial, antioxidant, and anti-damage pharmacological effects.[105–109] There are few independent studies on the anti-hepatocellular carcinoma of physcion, and most of them are studied in combination with anti-cancer drugs. In a mouse tumor model established by intraperitoneal injection of HepG2 cells, the study found that physcion (40 mg/kg/day and 20 mg/kg/day) inhibited the expression of specificity Protein 1 (Sp1) by enhancing the phosphorylation of AMP-activated protein kinase (AMPK) in the tumor tissue, thereby regulating the expression of DNA methyltransferase (DNMT1), ultimately mediating the expression of targeted miR-370 and inducing apoptosis through the mitochondrial pathway.[48] The expression of miR-370 in tumors is closely related to the death of liver cancer cells.[110] In addition, The MAPK enzyme family plays an important role in liver injury and tumorigenesis.[111,112] Molecular docking results show that physcion can bind to the sites of 4 MAPKs (P38, ERK2, JNK and MK3).[113] However, the anti-liver cancer mechanism of physcion-related MAPK has not been reported. FENG Q, etc., found that 6-phosphogluconate dehydrogenase activity (6PGD) expression is increased in different cancer tissues and can promote tumorigenesis.[114] The expression of 6PGD is involved in HCC growth and survival and is considered a potential target to overcome HCC resistance to chemotherapy drugs.[115] Physcion is an

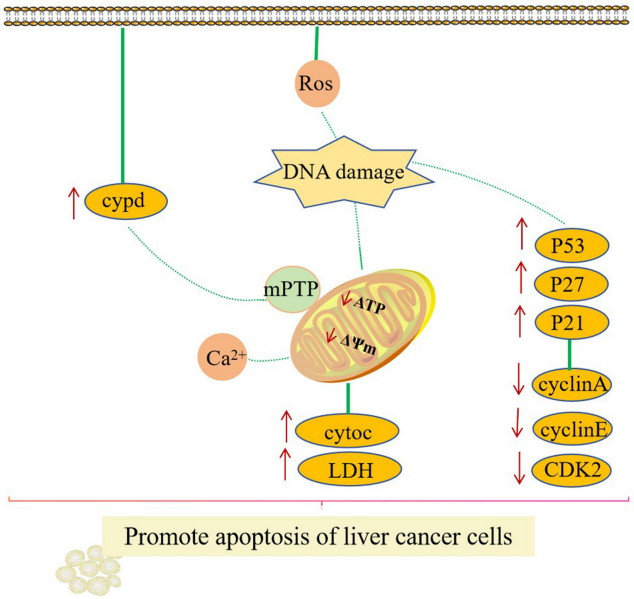


Figure 4 The molecular mechanism of chrysophanol anti -liver cancer.

inhibitor of the small molecule 6PGD. nicotinamide adenine dinucleotide phosphate (NADP) can be quickly converted into reduced nicotinamide adenine dinucleotide phosphate (NADPH) by G6PD. Cancer cells need to increase NADPH levels to increase nucleotide synthesis and prevent ROS production. Therefore, elevated NADPH levels are critical for the growth and survival of cancer cells.[116] Moreover, Chen H et al confirmed that 6PGD inhibition activates AMPK and acetyl CoA carboxylase 1 (ACC1), thereby reducing the NADPH/NADP + ratio and NADH levels in HCC cells. Furthermore, 6PGD inhibition significantly reduced NADP-dependent sirtuin 1 (SIRT1) enzyme activity, and the reduction of SIRT1 activity inhibited the growth and survival of HCC cells.[115] Glycolysis was first discovered in HCC cells and is a hallmark of liver cancer, responsible for regulating HCC cells proliferation, immune evasion, invasion, metastasis, angiogenesis, and drug resistance.[117] Pan X P et al's study found that physcion (20 μM) could enhance the inhibitory effect of sorafenib on drug-resistant HCC cells, increase the level of miR-370 and downregulate the expression of proto-oncogene serine/threonine-protein kinase 1 (Pim-1), thereby inhibiting c- Myc-regulated glycolysis.[49] Another study confirmed that combined treatment with physcion and sorafenib significantly inhibited tumor growth in mice. In vitro experiments showed that the anticancer mechanism of physcion synergistically enhancing sorafenib was related to the inhibition of Neurogenic locus notch homolog protein 3/AKT (Notch3/AKT) signaling in HCC cells. In addition, physcion can also enhance sorafenib-induced apoptosis of HCC cells, and physcion significantly enhances sorafenib-induced caspase-3 cleavage, which is related to liver cancer cell apoptosis. Moreover, physcion can further reduce the expression of anti-apoptotic protein Bcl-2 and increase the expression of pro-apoptotic protein Bax in HCC cells.[118] The mechanism of physcion anti -liver cancer is shown in Figure 5.

The Anti-Liver Cancer Effect and Mechanism of Aloe-Emodin

Aloe-emodin (1,8-dihydroxy-3-(hydroxymethyl)anthracene-9,10-dione) is the main AQ component of the traditional Chinese medicine. Aloe-emodin has anti-cancer, anti-viral, anti-inflammatory, antibacterial, neuroprotective and hepato-protective pharmacological effects.[118–122] The anti-liver cancer effect of aloe-emodin is related to promoting the apoptosis and growth of liver cancer cells. In terms of promoting apoptosis in liver cancer cells, aloe-emodin (40 μM) induces apoptosis in HepG2 cells by causing significant and sustained activation of JNK. However, aloe-emodin has no effect on the phosphorylation/activation of p38 in HepG2 cells, In addition, aloe-emodin has specific toxicity to liver cancer cells, and the toxicity produced in normal cells is not obvious (including: human gingival fibroblasts, human lung fibroblasts, hematopoietic progenitor cells and primary astrocytes and fibroblasts).[53] Lin ML et al's study found that aloe-

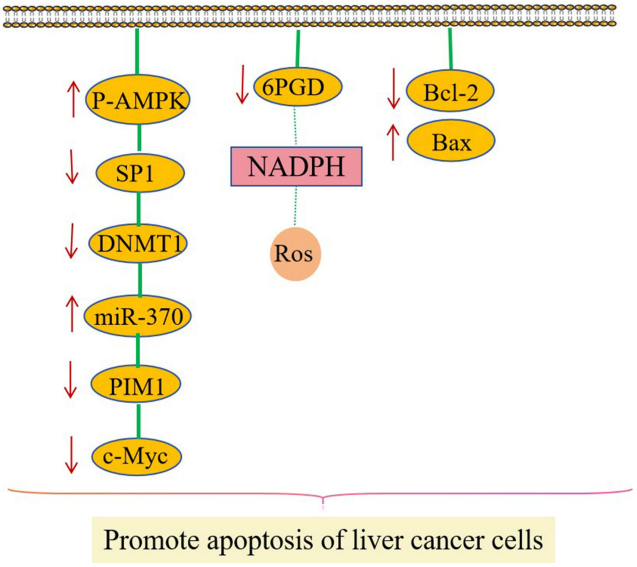


Figure 5 The molecular mechanism of physcion anti -liver cancer.

emodin (60 μM)-induced apoptosis in Hep3B cells is related to the apoptotic mechanism involving P53, mainly due to the decrease in cardiac ankyrin repeat protein (CARP) mRNA stability and subsequent induction of ERK and caspase-8-mediated mitochondrial death pathways. However, the molecular mechanism by which aloe-emodin reduces the stability of CARP mRNA in cells remains to be further explored.[54] Kuo P L showed that aloe-emodin (10 μg/mL, 20 μg/mL) has an inhibitory effect on both human liver cancer cell lines HepG2 and Hep 3B. aloe-emodin administration can arrest the HepG2 cell cycle in the G1 phase, its mechanism is related to aloe-emodin increasing the expression of p53 protein in HepG2 cells, thereby stimulating the expression of its downstream p21 protein. In addition, in p53-deficient Hep 3B cells, aloe-emodin promotes cell apoptosis by enhancing the expression of Bax.[50] Lu G D found that aloe-emodin is more cytotoxic than its analog emodin. For example, aloe-emodin can upregulate the expression of redox-sensitive proteins (peroxiredoxin-2, −4 (PRDX-2, −4) and parkinson disease protein 7 (PARK7/DJ-1)), suggesting that aloe-emodin can trigger oxidative stress in HepG2 cells. In addition, aloe-emodin inhibited DNA synthesis in HepG2 cells by upregulating the tumor suppressor p16 and inhibiting Retinoblastoma (Rb) phosphorylation, and reduced cell migration by upregulating the transfer of the inhibitor nonmetastatic protein 23 (nm23).[51] P-Rb plays an important role in controlling cell cycle progression and cell growth.[123] The PI3K-AKT signaling pathway is closely related to cell growth. Zhu M et al found that the PI3K-AKT signaling pathway involving AKT1 and EGFR is important in aloe-emodin treatment of HCC through network pharmacology. In vitro experiments also verified that after HCC cells were treated with aloe-emodin (50 μM, 100 μM), the expression of PI3KR1, AKT, and p-AKT proteins was down-regulated, confirming that aloe-emodin can promote apoptosis and inhibit proliferation of HCC cells through the PI3K-AKT signaling pathway.[52] The mechanism of aloe-emodin anti -liver cancer is shown in Figure 6.

Based on the above discussion, it can be found that the anti-liver cancer mechanism of AQs is extensive. The signaling pathways related to caspase, PI3K-AKT, MAPK and mitochondria-guided apoptosis can be extensively discussed. And 6PGD can be discussed as an important target of physcion. Modern research has confirmed that rhubarb free AQs (emodin, rhein, chrysophanol, emodin methyl ether, aloe-emodin) can regulate lipid metabolism by regulating the liver PPARα and SREBP target genes, PCSK9 and ABCG8 genes.[124] And some studies have shown that emodin and chrysophanol have strong effects on lipid metabolism, among which chrysophanol has a better lipid-lowering effect than emodin.[18] It is suggested that the anti-liver cancer mechanism based on the lipid-lowering effects of emodin and chrysophanol deserves in-depth study. Moreover, rhein has also been confirmed to exert anti-pancreatic cancer effects

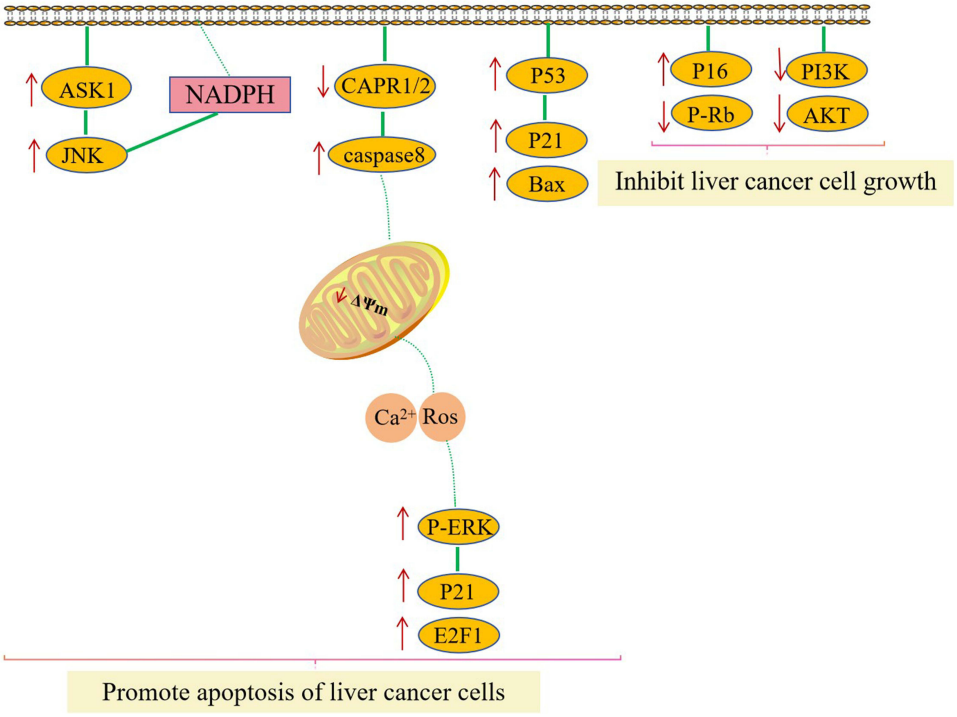


Figure 6 The molecular mechanism of aloe-emodin anti -liver cancer.

by inhibiting glycolysis.[125] Therefore, the study of AQ's involvement in anti-liver cancer through lipid regulation and glycolysis deserves further exploration.

Toxicity and Pharmacokinetics of Rhubarb AQs

With the widespread use of rhubarb, the toxicity of rhubarb has been paid more and more attention. The toxicity of rhubarb is mainly manifested in gastrointestinal toxicity, liver toxicity and nephrotoxicity.[26] AQs are considered to be the main toxic substances in rhubarb.[126] Rhubarb extract will cause obvious liver and kidney toxicity at high doses. The extract mainly contains 5 components, including emodin, rhein, chrysophanol, physcion, and aloe-emodin with contents of 0.900 mg/g, 0.639 mg/g, 3.506 mg/g, 0.780 mg/g and 0.304 mg/g respectively.[23] Infact, rhubarb extract has a bi-directional effect on the liver and kidneys. Studies have shown that rhubarb extracts (200, 600, 800 mg/kg for 6 weeks by gavage) exerted protective effects on the kidneys of rats,[127] whereas rhubarb extracts (40 g/kg for 5 weeks by gavage) caused toxicity in the kidneys of rats. Moreover, some studies have suggested that the nephrotoxicity of rhubarb is not purely dose-related, but that compositional factors are equally important. The order of nephrotoxicity of the main chemical components of rhubarb is as follows: Aloe-emodin > rhein > emodin>chrysophanol.[126] Therefore, the nephrotoxicity of rhubarb is greatly reduced after concoction.[127] Mechanism of nephrotoxicity of rhubarb AQs is associated with genes that improve oxidative stress, cell cycle and nutrient metabolism path ways.[128] In terms of hepatotoxicity, the study showed that rats receiving 10 g/kg for 12 weeks and 14.7 g/kg for 12 weeks (intraperitoneal injection) of rhubarb showed hepatoprotective effects, while 20 g/kg for 3 weeks and 32 g/kg for 5 days (by gavage) exhibited hepatotoxic effects.[129] It can be seen that dose control is an important factor in rhubarb AQs toxicity. Moreover, rhubarb AQs is capable of impairing mitochondrial function, ultimately leading to hepatotoxicity. The underlying mechanisms are related to cell cycle arrest, depolarization of mitochondrial membrane potential and inhibition of mitochondrial respiratory chain complex function.[130,131]

The toxicity of the main AQs in rhubarb are shown in Table 3. The toxicity of rhubarb AQs extract is mainly manifested in the liver and kidneys and is closely related to the dosage. Rats that received rhubarb extract (1.62 g/

Table 3 The Toxicity of Emodin, Rhein, Chrysophanol, Physcion, and Aloe-Emodin

Drug	Models	Dosage of Administration	Duration of Administration	Dosing Method	Toxicity Type	References
Emodin	Rat	150, 500, 1500 mg/kg	4 weeks	Oral	Hepatotoxicity	[132]
	Mice	0.8 and 1.6 g/kg	11 weeks	Oral	Nephrotoxicity	[133]
	Mice	1000 mg/kg	5days	Oral	Reproductive toxicity	[134]
Rhein	Mice	375 mg/kg/d	75days	Oral	Hepatotoxicity	[135]
	Mice	0.175 and 0.35g/kg	60days	Oral	Nephrotoxicity	[136]
	Mice	350, 175, 87.5 mg/kg	/	Oral	Cardiotoxicity	[137]
	Human colorectal cancer cells	40 μM	5days	Indicated	Colon toxicity	[138]
	Rat	87.5, 175 and 350mg/kg	10days	Oral	Reproductive toxicity	[139]
Chrysophanol	Rat hepatocytes	0, 1, 5, 25 and 50μM	24h	Indicated	Hepatotoxicity	[140]
Physcion	Human liver cell L-02	20 to 300 M	24h	Indicated	Hepatotoxicity	[141]
	Syrian baby hamster kidney (BHK-21) cells	20.69 ± 4.66 μg/mL	72h	Indicated	Nephrotoxicity	[142]
Aloe-emodin	OF1 mice	500, 1000 and 2000 mg/kg	3–6h	Oral	Hepatotoxicity Nephrotoxicity	[143]
	Human skin fibroblast cells	1 mM	30 min	Indicated	Phototoxicity	[144]

kg) orally had a significant increase in the relative weight of the spleen and kidneys, and the kidneys showed hydronephrosis, swelling and blackening, and the pigmentation of renal tubular epithelial cells increased significantly. However, no adverse reactions were observed in rats orally treated with 0.65 g/kg rhubarb AQs extract. In addition, the component analysis of the extract showed that the main components are aloe-emodin, emodin, rhein, chrysophanol and physcion.[145] Rats also showed mild hepatotoxicity after three weeks of administration of rhubarb extract (3 and 20 g/kg, respectively 6 and 40 times the maximum human dose (0.5 g/kg) specified in the Chinese Pharmacopoeia), High Performance Liquid Chromatography (HLPC) showed that its components also mainly include aloe-emodin, rhein, emodin, chrysophanol, physcion and danone.[146] In addition, Xing X's research also found that the hepatotoxicity of rhubarb extract will increase with the increase of rhubarb dosage, and the proportions of each component are aloe-emodin 0.24%, rhein 0.457%, emodin 0.502%, and chrysophanol 0.865%, physcion 0.235%.[147] Rhein may be the main toxic substance of rhubarb AQs. A study analyzed the cytotoxicity of five AQs on rat liver cells and found that rhein is the most toxic component, followed by emodin, aloe-emodin, physcion and chrysophanol.[148] Many studies have also confirmed that rhein can cause multi-organ toxicity. For example, Cheng Y found that long-term use of rhubarb AQs can cause colon toxicity and increase the risk of colon cancer. And it was found that the toxic metabolite that causes escherichia coli melanosis is mainly rhein in rats that took rhubarb AQs for 90 days. In addition, the fecal flora of rats can bioconvert sennoside A into rhein. In vitro experiments showed that the mechanism of chronic toxicity of rhubarb AQs is related to the induction of apoptosis and autophagy in human colorectal cancer cells by rhein (10 μM, 40 μM).[138] Moreover, Liu Y et al combined the effects of cytotoxicity, plasma concentration and daily intake to determine the hepatotoxicity of 16 AQs and their derivatives. The results showed that the toxic dose of rhein is much lower than that of emodin and aloe-emodin, it is suggested that rhein is a potentially hepatotoxic substance. This result also suggested that even though many traditional Chinese medicines contain AQs, taking large doses of rhubarb requires attention to the risk of liver toxicity because rhein is the main component of rhubarb.[149] Similarly, rhein (0.175 g/kg/day, 0.35 g/kg/day) can cause chronic poisoning in mice, manifested in liver damage with abnormal expression of aspartate aminotransferase (AST), alanine aminotransferase (ALT), interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α) levels, which is consistent with rhein induces oxidative stress and is associated with induction of mitochondrial dysfunction and activation of apoptosis. In addition, large doses of rhein (4000 mg/kg and 2000 mg/kg) can also cause poisoning in mice, with varying degrees of damage to liver tissue, kidney tissue and colon tissue.[135] Rhein has also been confirmed to be cardiotoxic. For example, rhein (350, 175, 87.5 mg/kg) can reduce the left ventricular ejection fraction (LVEF) and leftventricular fractional shortening (LVFS) of cardiac function, and upregulate the levels of Ca2+, cardiac troponin T (cTnT), creatine Kinase (CK) and LDH in the serum of mice. The mechanism involved in the cardiotoxicity of rhein is related to the fas-induced apoptosis pathway.[137] Emodin may also be the toxic component of rhubarb AQs. The expression of hepatocyte nuclear factor 4 (Hnf4α) and uridine diphosphate glucuronosyltransferase (UGT2B7) in HepG2 cells and rats was reduced and caused hepatotoxicity after emodin treatment.[150] In addition, emodin (25–75 μM) can induce blastocyst damage in mice through intrinsic apoptotic signaling, thereby affecting embryonic development.[151] Yang W et al administered rhubarb extract (1620 mg/kg) to rats for 52 weeks, its main components include aloe-emodin, emodin, rhein, chrysophanol, and physcion. The results showed that the nephrotoxicity of rhubarb extract was manifested as damage to renal tubular epithelial cells, suggesting that emodin may be the main toxic component of nephrotoxicity.[23] Similarly, Luyong Z et al also showed that the toxic target organ of rhubarb AQs in rats should be the kidney (especially the proximal renal tubules).[152] However, both emodin and rhein have been shown to induce apoptosis in human proximal renal tubular epithelial cells,[153] which indicates that rhein may also be the main substance causing nephrotoxicity. Therefore, the author believes that there is insufficient evidence that emodin is the main hepatotoxic component in rhubarb AQs. In fact, the nephrotoxicity and hepatotoxicity of aloe-emodin have also been reported. Aloe-emodin can induce primary DNA damage in the liver and kidneys of mice, suggesting that it may have certain hepatotoxicity and nephrotoxicity.[143] And aloe-emodin reduced the viability of HK-2 cells (human proximal renal tubular epithelial cell line) in a dose- and time-dependent manner and induced HK-2 cells to arrest in the G2/M phase, this is related to ERS induced by aloe-emodin.[154] The hepatotoxicity of physcion is weaker than that of emodin, and physcion and

emodin show moderate and severe cytotoxicity to the human liver cell line L-02, respectively.[141] In fact, there are few studies on the toxicity of chrysophanol and physcion, and almost all of them are in vitro experiments. Therefore, the toxicity of these two components still deserves in-depth study. Therefore, it is questionable to directly define all rhubarb AQs as hepatotoxic or nephrotoxic substances. In addition, aloe emodin glycoside and physcion glycoside among the AQ glycosides are also considered to be toxic substances of rhubarb AQs. Wang J B believed that the liver and kidney toxicity of the total AQ glycosides in rhubarb are greater than the total AQs. The liver toxicity and kidney toxicity of processed rhubarb are reduced compared with raw rhubarb.The results of content testing showed that its attenuating effect may be related to the decrease in the content of aloe emodin glycoside and emodin glycoside.[126] In summary, the most toxic of the five common rhubarb AQs is rhein, followed by emodin and aloe-emodin. However, the toxicity of chrysophanol and physcion deserves further study. In addition, the toxicity of AQ glycosides needs to be studied.

Pharmacokinetics plays a guiding role in the clinical application of rhubarb AQs and can be used to evaluate the safety and effectiveness of medications.[157] The pharmacokinetics of rhubarb AQs are commonly tested by HPLC and LC-MS.[156,158] AQs have high fat solubility, are mainly absorbed in the intestine, and then distributed in organs and tissues with rich blood flow, such as intestines, stomach, liver, lungs, kidneys, fat, etc. The main metabolic pathways include intestinal flora and liver, excretion pathways are kidneys, rectum and gallbladder. The blood concentration of AQs will fluctuate due to enterohepatic circulation, reabsorption and conversion.[159] The pharmacokinetic parameters of rhubarb AQs(mainly including aloe-emodin, rhein, emodin, chrysophanol and physcion) are shown in Table 4. LC-MS was used to detect the distribution of free AQs in rat tissues after oral administration of total rhubarb extract. The results showed that five free AQs, aloe-emodin, rhein, emodin, chrysophanol and physcion were detected in the liver, kidney and spleen of rats. The contents of rhein, aloe-emodin and emodin has reached limit, the content of components in kidney and spleen tissue is rhein > aloe-emodin > emodin, and the content of components in liver tissue is aloe-emodin > rhein > emodin. Free AQs is not detected in tissues several weeks after drug withdrawal.[160] Similarly, rats were orally administered total rhubarb extract (14.49 g/kg) for 12 weeks, and the concentration of AQs in different tissues was quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). It was found that five major AQs (aloe-emodin, rhein, emodin, chrysophanol, and physcion) could be detected in the liver, kidney, and spleen of rats. Only rhein, aloe-emodin, and emodin reached the quantitation limit, the contents of the three AQs in the same tissue were rhein > emodin > aloe-emodin, and 4 weeks after drug withdrawal, no AQs were detected in these tissues, indicating that rhubarb has almost no cumulative toxicity.[161] Moreover, there are reports that a woman developed acute renal failure after taking rhubarb AQs derivatives and recovered automatically after stopping taking the medicine.[162] This also suggests that rhubarb AQs may not have cumulative toxicity. According to pharmacokinetic results, rhein, aloe-emodin and emodin are deposited more in the liver, kidney and spleen, which also suggests that rhein, aloe-emodin and emodin are the main toxic substances of rhubarb AQs, and based on the above-mentioned toxicity studies and pharmacokinetic results, we can find that rhein may exist as the main toxic component of rhubarb AQs. It was mentioned that in vitro metabolism studies of intestinal and liver microsomal proteins in rats and humans showed that the systemic exposure of free rhein is 20 times greater than that of free aloe-emodin and emodin, and the activity of rhein significantly higher than the other four AQs, which may be related to the higher solubility and poorer glucuronidation of rhein, which also suggests that rhein is the main toxic component of rhein AQs.[163] In addition, the corresponding glucosides of emodin, rhein, aloe-emodin, chrysophanol, and physcion were not detected in the plasma after rats took rhubarb extract.[163] Similarly, Song R et al also found that 11 of the 12 metabolites transformed by the intestinal flora of rats were AQ metabolites, including bioconverting the corresponding glucosides of aloe emodin, rhein, emodin, chrysophanol, and physcion into the corresponding aglycones.[164] This also reveals why there are less studies on anti-liver cancer and toxicity research on rhubarb AQs glycosides. Moreover, the impact of AQs on their toxicity based on the metabolism of intestinal flora still needs to be studied. In fact, pharmacokinetics is not only related to factors such as plasma volume, gastric emptying time, plasma protein levels, cytochrome P450 activity, drug transporter function and excretion activity,[165] but also related to factors such as gender and body weight.[166] Therefore, it is meaningful to bring the above factors into the toxicity study of rhubarb AQs.

Table 4 The Pharmacokinetic Parameters of Emodin, Rhein, Chrysophanol, Physcion, and Aloe-Emodin

composition	Route of Administratio	Species	Dose (a:g/kg:b: mg/kg: Equivalent to Rhubarb Crude Drug)	Pharmacokinetic Parameters									References
				Tmax (h)	AUC0-t (a: μg.h/mL;b: mg.h/L)	AUC0-(a:μg.h/ mL;b:mg.h/L)	t1/2 (h)	Vd (L/kg)	Cl (L/h/kg)	Cmax (a:μg/ mL;b:mg/l)	MRT0-t (h)	MRT0- (h)	
Alo-emodin	Oral	Rat	5(a)	0.47 ±0.15	1.24 ±0.09(a)	1.59 ±0.16(a)	10.54 ±0.21	248.5 ±0.10	16.78 ±0.17	0.18 ±0.11(a)	8.03 ±0.05	14.76 ±0.20	[155]
Rhein				0.50 ±0.00	17.79 ±0.21(a)	20.30 ±0.30(a)	6.80 ±0.41	16.38 ±0.16	1.83 ±0.30	5.01 ±0.16(a)	7.12 ±0.11	10.14 ±0.33	
Emodin				0.47 ±0.15	2.46 ±0.26(a)	2.95 ±0.32(a)	9.05 ±0.26	319.0 ±0.27	25.27 ±0.26	0.48 ±0.25(a)	7.65 ±0.08	12.33 ±0.25	
Chrysophanol				0.75 ±0.00	1.49 ±0.23(a)	1.92 ±0.34(a)	10.86 ±0.48	649.2 ±0.28	44.74 ±0.30	0.22 ±0.18(a)	8.14 ±0.12	15.08 ±0.45	
Physcion				0.70 ±0.14	1.57 ±0.35(a)	2.19 ±0.41(a)	12.91 ±0.19	489.9 ±0.30	27.35 ±0.36	0.26 ±0.22(a)	8.10 ±0.09	17.51 ±0.19	
Alo-emodin	Oral	Beagle dogs	150(b)	0.75 ± 0.11	0.35 ± 0.058(b)	0.42 ± 0.083(b)	14.73 ± 6.52	1309.86 ± 555.46	61.63 ± 10.47	0.031 ± 0.005(b)	/	/	[156]
Rhein				1.50 ± 0.20	32.22 ± 8.29(b)	35.15 ± 10.23(b)	10.11 ± 2.29	14.33 ± 3.03	0.98 ± 0.22	3.39 ± 0.43(b)	/	/	
Emodin				0.75 ± 0.16	2.97 ± 0.66(b)	4.05 ± 0.94(b)	18.73 ± 3.82	462.72 ± 123.65	17.12 ± 5.53	0.27 ± 0.061(b)	/	/	
Chrysophanol				1.00 ± 0.25	0.43 ± 0.10(b)	0.54 ± 0.14(b)	15.18 ± 2.92	3210.97 ± 800.29	146.61 ± 47.18	0.036 ± 0.009(b)	/	/	
Physcion				2.00 ± 0.26	0.41 ± 0.12(b)	0.48 ± 0.14(b)	13.08 ± 2.19	2067.15 ± 656.96	109.53 ± 33.36	0.032 ± 0.006(b)	/	/	

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Discussion

After consulting a large amount of literature, we found that many components of rhubarb AQs have good therapeutic effects on liver cancer. The main anti-liver cancer components include emodin, rhein, chrysophanol, physcion and aloe-emodin. The anti-liver cancer mechanism of rhubarb AQs mainly involves promoting the apoptosis of liver cancer cells, inhibiting the growth of liver cancer cells, and synergistically enhance the sensitivity of chemotherapy drugs. Promoting apoptosis of liver cancer cells involves mitochondrial apoptosis, caspase enzyme activation, regulation of cancer cell cycle, lipid metabolism and glycolysis, while inhibiting cancer cell generation involves the autophagy pathway and PI3K-AKT pathway. In fact, rhubarb AQs have long been studied for the treatment of liver diseases such as NAFLD,[167] acute liver injury,[168] the treatment involves improving liver function levels, anti-inflammation, and anti-oxidative stress, etc. It can be seen that rhubarb AQs can be widely studied as a drug acting on the liver. Although the anti-liver cancer effects of rhubarb AQs have been widely studied, there is still a lack of research on the anti-liver cancer effects of rhubarb total AQs/rhubarb combined AQs. Therefore, research on the anti-liver cancer of rhubarb total AQs/rhubarb combined AQs can be increased and screen out AQs with better anti-liver cancer activity. Yuan Y et al incubated rhein with different types of liver microsomes and identified the metabolites of rhein as two hydroxyglucuronides and one acyl glucuronide. It was found that rhein acyl glucuronide is chemically reactive and exhibits cytotoxicity to liver cancer cells.[169] However, the current studies on the anti-liver cancer effects of rhubarb AQs are based on in vitro experiments, there is a lack of research on the anti-liver cancer effects of rhubarb AQs metabolites, so it is recommended to increase in vivo research on the anti-liver cancer effects of rhubarb AQs.[170] In addition, TCM meridian science believes that vinegar can introduce medicine into the liver meridian. The combination of rhein and bupleurum processed with vinegar is a simple and effective liver-targeted treatment method,[170] one of the ways to prepare rhubarb is vinegar. Therefore, it is meaningful to use the AQs extracted from vinegar-based rhubarb to fight liver cancer or treat liver disease. In addition, several studies on rhubarb AQs have shown that its synergistic anticancer drugs and chemotherapy can enhance the efficacy against hepatocellular carcinoma, so the combined efficacy of rhubarb AQs against cancer can be explored in the future. Moreover, due to the complex transformation of AQs in vivo, the mechanism of efficacy remains to be investigated, so it is recommended to study the single component.

Modern research generally believes that rhubarb AQs is toxic. There is no cumulative effect on the toxicity of rhubarb anthraquinone, but the dose, regimen, and composition are all related to its toxicity, suggesting that if rhubarb anthraquinone is used for a prolonged period of time, it should be monitored at all times and discontinued if toxicity occurs. Liu C believed that rats did not experience toxic reactions when taking 0.65 g/kg of rhubarb extract orally, however, rats showed toxic effects when the dose reached 1.62 g/kg.[145] In acute and subchronic toxicity experiments of oral AQs in rats, the acute toxicity study showed that the LD50 was >5 g/kg. In a 90-day subchronic toxicity study, when the oral dose reached 5.44 mg/kg, clear droplets accumulated in the renal tubules of male rats, and female rats showed anemia. When the oral dose reached 174.08 mg/kg, the hepatocytes around the central vein of the liver lobule in female rats were slightly hypertrophied and the thyroid function was reduced.[171] Therefore, controlling the dose of rhubarb AQs is the key to toxicity. Although modern research believes that large doses of rhubarb extracts can cause liver toxicity, there is a lack of an anti-liver cancer dose of rhubarb AQs in animals. The continued research is needed to find a dose range that can promote the apoptosis of liver cancer cells without damaging normal liver cells. Furthermore, although existing research suggests that rhubarb AQs are hepatotoxic, the hepatotoxicity of rhubarb has rarely been studied. However, the hepatotoxicity of Polygonum multiflorum (PM) containing AQs has been widely reported. In a study, it was found that the hepatotoxic components in PM are mainly emodin-8-O-β-D-glucoside, emodin and stilbene glycoside.The combination of emodin and stilbene glycoside will produce greater hepatotoxicity.[172] It is suggested that the toxic effects of AQs may be enhanced when combined with other components. In addition, the combination of AQ-containing drugs with nonsteroidal drugs can induce hepatitis and acute renal failure. Therefore, hepatotoxic and toxic drugs should not be combined with AQs to avoid additive effects.[173] The use of new drug delivery technologies and improved dosage forms can be the key to attenuating the toxicity of rhubarb AQs. Colon-targeted drug delivery can ensure that the drug is directly treated at the disease site, and can also reduce dosage and systemic side effects.[174] Zhang L et al applied colon-specific drug delivery technology (CDD-GN) to AQ aglycone, which can be specifically released in the colon after oral

administration. This technology not only retains the purgative effect of AQs, but also prevents AQs from being absorbed in the intestines and promotes excretion, thereby greatly reducing the toxicity of rhubarb AQs. Moreover, the pharmacokinetic showed that the AUC, Cmax, Tmax, and t1/2 of aloe-emodin, rhein, emodin, and chrysophanol in rats receiving CDD-GN were significantly reduced.[175,176] And the reduced systemic exposure of aloe-emodin, emodin, and physcion in the RTFA-OCDD-GN group may help alleviate the nephrotoxicity caused by rhubarb.[177] Hydrogel is a therapeutic agent that provides sustained, local delivery, using chitosan as a scaffold material in the hydrogel with good biocompatibility, low toxicity and biodegradability.[178] Hydrogel may be an ideal carrier for rhein. Making rhein and chitosan into a hydrogel can reduce the toxicity of rhein, and the gel shows a high degree of biocompatibility.[179] In addition, rhubarb AQs exists as a whole with multiple components, and there will be interactions between substances, which may help to reduce its toxicity. In human intestinal Caco-2 cells, aloe emodin and physcion were used together with emodin to significantly reduce the absorption of emodin, suggesting that emodin may be more effective and safer when mixed with other free rhubarb total AQs (TRA).[180] It is suggested that the comparison of the toxic effects of using rhubarb AQs multi-component synergistically and using a single component can be a future research direction. In fact, other traditional Chinese medicines used together with rhubarb AQs will also reduce the blood concentration of toxic components of rhubarb AQs. For example, compared with taking rhubarb alone, when rhubarb and aconite are taken orally together, the Cmax of rhein will be greatly reduced.[181] This shows that rhubarb AQs can also interact with other components in traditional Chinese medicine to reduce toxicity.

In terms of bioavailability, one interesting study showed that in rhubarb extracts, only rhein was absorbed by the body, even though the levels of aloe-emodin and emodin, chrysophanol were tens or hundreds of times higher than rhein levels. But only rhein is absorbed by the body.[182] The bioavailability of rhein, emodin and chrysophanol were comparable,[183] while the bioavailability of aloe-emodin was the lowest. This may be due to the fact that aloe-emodin can be oxidized to rhein in the body.[184] Some studies have indicated that poor solubility and low bioavailability have limited the use of rhein. Instead, rhein derivatives are more effective in the treatment of various diseases in vitro and in vivo, so nanotechnology-based drug delivery systems can be considered to improve the delivery barrier of rhein and significantly increase its bioavailability.[185]

In summary, rhubarb AQs can be used as a potential anti-liver cancer drug, but its research still has many limitations. For example: rhubarb AQs has many components, but only a few of them have been used in anti-liver cancer research. There is a lack of in vitro testing and a lack of research on the anti-liver cancer effects and mechanisms of rhubarb AQs metabolites. Moreover, the toxicity of rhubarb AQs is also an obstacle to anti-liver cancer research. Therefore, when conducting research on rhubarb AQs, it is recommended that the toxicity of rhubarb AQs should be reduced by avoiding combination with components of the same toxicity, improving the dosage form, reducing the content of AQ glycosides, and using green solvents for extraction, in order to reduce the toxicity of rhubarb AQs. In the future, there are still some challenges in researching and developing rhubarb AQ as a clinical drug, (1) the complexity of the composition and the difficulty in controlling the quality. (2) Inadequate safety evaluation. (3) Insufficient research on drug efficacy, pharmacokinetics, and mechanism, which increases the difficulty of setting up clinical trial protocols. Potential strategies: (1) Gradually clarify the single component, and gradually clarify the therapeutic mechanism of the component. (2) Clarify its toxicological mechanism. (3) Explore new types of formulations so as to improve their efficacy and reduce side effects.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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