Protective Effects of Pomegranate and Its Bioactive Components on Hepatic Disease

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Abstract By summarizing the pharmacological effects of pomegranate extract and its active components, such as punicalagin, punicalin, gallic acid, ellagic acid, caffeic acid, and chlorogenic acid, it is found that the extract exhibits therapeutic effects on liver injury, viral hepatitis, metabolic dysfunction-associated fatty liver disease, liver fibrosis, and liver cancer. Emerging evidence suggests that these natural products may alleviate liver diseases through multi-targeted therapeutic mechanisms, including anti-inflammation, anti-oxidative stress, immunoregulation, and anti-steatosis. The underlying mechanisms by which pomegranate exerts hepatoprotective activities may be attributed to the regulation of multiple signaling pathways, including P62/Nrf2, TGF-β1/Smad7, Wnt/β-catenin, MAPK/Nrf2, Nrf2/Keap1, Akt/FOXO3a, MAPK/NF-κB, *etc*. Consequently, pomegranate can serve as a functional food, nutritional supplement, or adjuvant in the modern treatment of liver diseases.

Key words Pomegranate, Hepatic disease, Hepatoprotective, Functional food, Mechanism of action, Pharmacology effect

1 Introduction

Chronic liver diseases are the prime cause of death and disability worldwide[1]. The etiology of liver diseases is multifactorial, encompassing a variety of causative agents. Predominant among these agents are xenobiotics, which include alcohol, drugs, and chemicals, as well as microorganisms, metabolic diseases, and autoimmune diseases[2]. Hepatic diseases can be classified into several categories based on their etiologies, including metabolic dysfunction-associated fatty liver disease, viral hepatitis, cholestatic liver diseases, and autoimmune liver diseases[3]. Currently, drugs such as silymarin and colchicine are recognized for their therapeutic potential in the management of liver injury. Nevertheless, these medications are associated with a range of adverse effects, which may include nausea, diarrhea, rash, itching, and breathing difficulties. Silymarin exhibits low oral bioavailability and undergoes significant first-pass metabolism in the liver, which limits its applicability in clinical trials[4]. Consequently, there is a pressing need for natural agents that are effective while minimizing the risk of adverse side effects. Meanwhile, numerous studies have demonstrated the beneficial effects of pomegranate in reducing hepatic injury[5-6]. Therefore, pomegranate may serve as an appropriate candidate for alternative medicinal therapies or dietary supplements.

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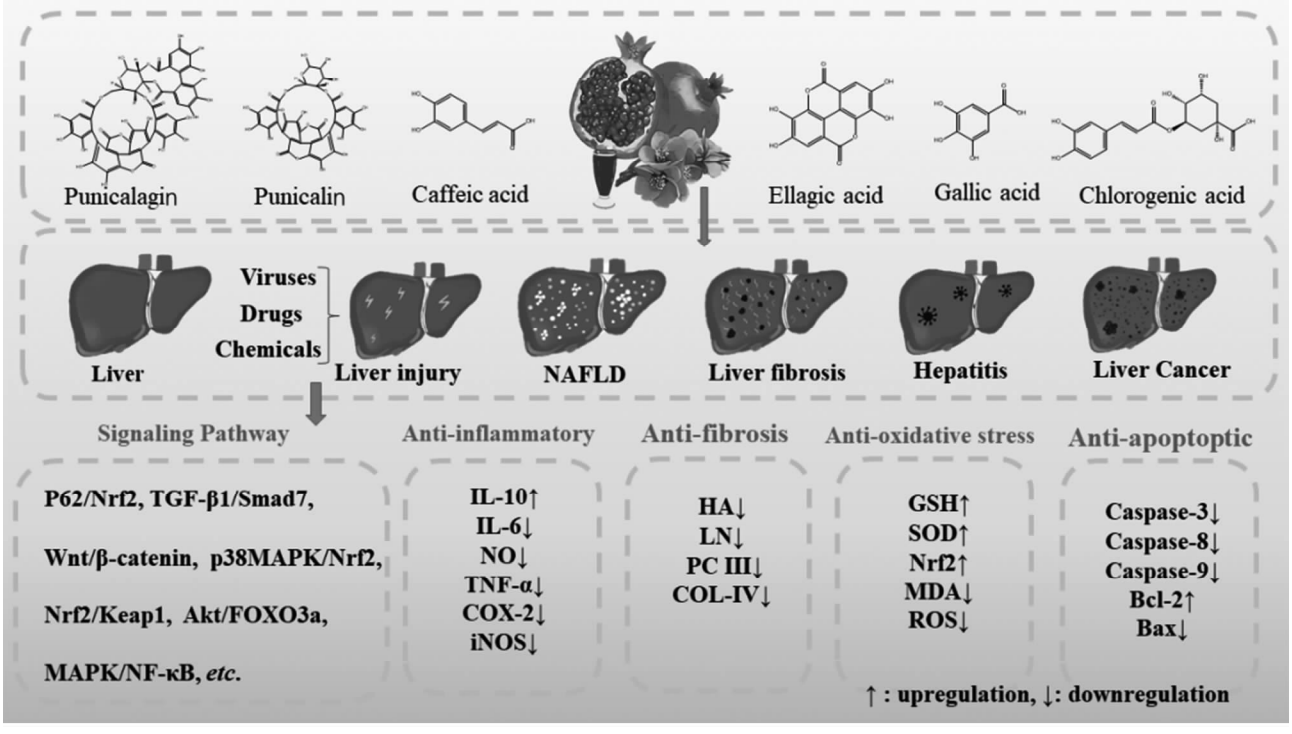
Pomegranate, a member of the Punicaceae family, is recognized as a functional food owing to its wide range of biological functions[7]. This fruit is believed to have originated in Central Asia, including ancient Persia and Afghanistan[8]. Currently, it is cultivated in several regions, including the Middle East, Asia, Southern Europe, the United States, and the temperate climatic zones of Africa[8]. Pomegranate has been used in medicine, food, and other fields, with its medicinal properties documented in the *Compendium of Materia*. Historical records indicate that the seeds, flowers, peel, and juice of pomegranate have been employed for medicinal purposes, such as hemostasis, insect repellent, and antidiarrheal treatments[9]. Recent research on the components of pomegranates has garnered increasing attention in the fields of nutrition and medicine. Modern pharmacological studies have found that pomegranate extracts and their components possess numerous beneficial effects, including the prevention and/or treatment of cancer, nerve damage, inflammation, ulcers, diabetes, dental diseases, hypercholesterolemia, cardiovascular diseases, obesity, and bacterial infections[10].

Pomegranate extracts and their active component have been shown in recent studies to have a range of hepatoprotective effects on various liver diseases, including drug-induced liver injury, chemically induced hepatotoxicity, viral and non-viral hepatitis, non-alcoholic fatty liver disease (NAFLD), liver fibrosis, cirrhosis, and liver cancer, among others. This review seeks to provide a comprehensive summary of the hepatoprotective effects and potential mechanisms associated with pomegranate extract and its primary components, hoping to give strategic support that may facilitate the application of pomegranate in the clinical management of liver disease.

2 Hepatoprotective active components of pomegranate

Fig. 1 shows that natural products found in pomegrante may exert a protective effect against various types of liver diseases through multiple mechanisms. So far, many studies have shown that over

500 compounds have been identified from various parts of pomegranate, including the fruit, juice, peel, seeds, flowers, leaves, and bark[11]. Currently, a total of 88 ellagitannins, 171 free and conjugated forms of flavonoids, 124 phenolic acids, and 74 hydroxycinnamic acids have been isolated and identified from various parts of pomegranate. Flavonoids, ellagitannins, and phenolic acids are the most prevalent compounds found in pomegranate juice, peel, flowers, and seeds[12]. Among these, hydrolysable tannins, phenolic acids, and flavonoids are regarded as the primary active components of pomegranate. The phenolic compounds of pomegranate include hydrolysable tannins (punicalin, punicalagin, pedunculagin), flavonoids (catechin, epicatechin, quercetin, rutin, kaempferol, hesperidine, anthocyanins, procyanidins), and phenolic acids (gallic, ellagic, vanillic, caffeic, ferulic, cinnamic, p-coumaric acids), among others[5,13]. Various monomeric compounds found in pomegranate, including punicalagin, punicalin, caffeic acid, gallic acid, ellagic acid, and chlorogenic acid, have been confirmed to have significant hepatoprotective activity both *in vitro* and *in vivo*.



NOTE IL-10. Interleukin-10; IL-6. Interleukin-6; NO. Nitric oxide; TNF-α. Tumor necrosis factor-alpha; iNOS. Inducible nitric oxide synthase; COX-2. Cyclooxygenase 2; GSH. Glutathione; SOD. Superoxide dismutase; Nrf2. Nuclear factor E2-related factor 2; MDA. Malondialdehyde; ROS. Reactive oxygen species; HA. Hyaluronicacid; LN. Laminin; PC III. Precollagen type III; COL-IV. Collagen IV; Bax. BCL2-associated X; Bcl-2. B-cell lymphoma-2.

Fig. 1 Pharmacological effects of natural products in pomegranate and related liver diseases

3 Pharmacological roles of pomegranate and its active components in liver injury

Liver injury can result from a variety of factors, such as drugs, chemicals and viruses. Drug-induced liver injury specifically occurs when a drug or its metabolites induce abnormal liver function, which may manifest as hepatocyte toxicity or liver injury caused by allergic reactions during treatment[14-15]. Pomegranate and its active components have been shown to improve liver damage through multiple pathways.

3.1 Extracts

3.1.1 Peel extract. Vancomycin (VM) is a glycopeptide antibiotic known for its bactericidal activity against Gram-positive bacteria. However, it is important to note that vancomycin may also induce hepatotoxic side effects[16]. El *et al.*[17] found that pomegranate peel ethanol extracts (PPEE) reduced the VM-induced hepatotoxicity. PPEE has the potential to regulate the Nrf2-ARE signaling pathway, thereby combating the reactive oxygen species (ROS) environment and consequently detoxifying and mitigating the negative consequences of ROS formation. It was also observed that PPEE reduced VM-induced toxicity by hindering apoptotic cell death through the regulation of apoptosis-related protein expression.

Studies have shown that pomegranate peel extract can also reduce liver damage caused by acrylamide and toluene (TOL) through the reduction of liver inflammation, alleviation of oxidative stress, and regulation of essential proteins[18-19].

3.1.2 Seeds oil extract. Pomegranate seed oil has a high content of phytosterol and a unique fatty acid profile[20]. Punicic acid, lin-

oleic acid, and oleic acids are the most abundant fatty acids found in pomegranate seed oil. Notably, punicic acid, a conjugated linolenic acid isomer, is a conjugated fatty acid composed of 18 carbons atoms and three double bonds, and it constitutes the main component of pomegranate seed oil[21].

Diethylnitrosamine (DEN) is a prototypical compound within the class of carcinogenic N-nitroso compounds. Administration of DEN to animals has been shown to cause injury to the liver[22]. Mohamed *et al.*[23] demonstrated the protective effects of pomegranate peel methanol extracts (PPME) and seed oil n-hexane extracts (PSOE) against DEN-induced hepatic injury in rats. This protective effect was validated by a considerable improvement in liver function tests. In addition, both PPME and PSOE possess antioxidant and radical scavenging properties, which contribute to the attenuation of programmed cell death.

Previous studies have indicated that CDDP-induced liver damage primarily presents as sinusoidal dilatation and congestion, hepatocellular degeneration, and inflammatory infiltrates within the liver[24]. In a study involving rats, the oral administration of pomegranate seed extract (PSE) at a dosage of 300 mg/kg for 15 consecutive days demonstrated hepatoprotective effects against the toxic impact of CDDP. Furthermore, treatment with PSE increased the activities of glutathione (GSH), total glutathione peroxidase (t-GPX), and superoxide dismutase (SOD)[25].

3.1.3 Juice extract. A study demonstrated that the oral administration of pomegranate juice (PJ) at a dose of 1mL via gavage for 5 weeks decreased sodium fluoride toxicity in the liver, as evidenced by improvements in both functional and histopathological parameters. Furthermore, PJ was found to scavenge NaF-induced free radical production, increase antioxidant defenses, and attenuate hepatic susceptibility to oxidative stress[26].

3.1.4 Flowers extract. Studies have shown that pomegranate flower extract can reduce liver injury induced by trichloroacetic acid (TCA) and Fe-NTA. Biochemical analysis showed that treatment with pomegranate flower extract resulted in a reduction of serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Histopathological evaluation revealed that pretreatment with pomegranate flower extract reduced balloon degeneration, fat change and necrosis[27-28].

3.1.5 Peel polysaccharides. The polysaccharide isolated from pomegranate peels is recognized for its outstanding antioxidant, hepatoprotective, and immunomodulatory properties[29]. This polysaccharide exhibits a neutral pH and demonstrates high solubility in water. It is classified as a galactomannan with a molecular weight of 110 kDa, characterized by a β-1→3 galactopyranose backbone and side chains composed of β-D mannopyranose and α-D mannopyranose[30].

Persistent exposure to carbon tetrachloride (CCl_4) induces cellular necrosis, oxidative stress, and inflammation, thereby affecting the morphology and function of the liver[31]. *In vitro* tests demonstrated that pomegranate peel polysaccharides (PPP) exhibited outstanding reducing power and scavenging effects against free radicals. The administration of PPP in mice significantly mitigated the elevated serum levels of ALT, AST, alkaline phosphatase, and hepatic MDA in CCl_4-induced mice. Additionally, the hepatic enzymatic activities of SOD, glutathione peroxidase (GSH-px), catalase, and non-enzymatic activity of glutathione were increased at 200 mg/kg · bw of PPP[32]. Hence, PPP demonstrates a significant potential to mitigate the liver injury induced by CCl_4.

3.2 Monomer

3.2.1 Punicalagin. Punicalagin is an ellagitannin that is found in the peel of pomegranate. This polyphenol has demonstrated antioxidant, hepatoprotective, anti-atherosclerotic, and chemopreventive activities.

Studies have shown that punicalagin has a protective effect on liver injury caused by CCl_4 and cyclophosphamide (CYP). Punicalagin alleviates CCl_4-induced liver injury by mediating the Akt/FOXO3a and P62/Nrf2 signaling pathways, which in turn activate autophagy[33] Another study showed that punicalagin protected rat liver by inhibiting oxidative and nitrosative stress, inflammation, and apoptosis, thereby reducing the toxic effects of cyclophosphamide[34].

3.2.2 Punicalin. Punicalin is a kind of ellagitannin that exists in pomegranate husks and has shown remarkable biological activities[35]. In the study conducted by Lin *et al.*[36], punicalin demonstrated anti-hepatotoxic activity against acetaminophen-induced toxicity in the liver of rats. Similarly, the findings indicate that punicalin has anti-hepatotoxic activity against CCl_4-induced toxicity in liver. Punicalin was found to reverse histological changes around the hepatic central vein and oxidative damage induced by acetaminophen and CCl_4[36-37].

3.2.3 Ellagic acid (EA). EA is a bioactive polyphenolic compound that occurs naturally as a secondary metabolite in various plant taxa. The EA content is particularly high in pomegranate, where it is predominantly located in the pericarp, bark, seeds, and flowers[38]. Structurally, EA is a dilactone of hexahydroxydiphenic acid, which is a dimeric derivative of gallic acid, produced mainly through the hydrolysis of ellagitannins[39]. EA is the major active compound of pomegranate and exhibits a range of beneficial properties, including antimutagenic, anti-inflammatory, antifibrotic, anti-cancer, and anti-aging effects[40].

Recent studies have demonstrated that EA possesses antioxidant and cytoprotective properties, which can effectively prevent lipid peroxidation and subsequently protect the liver from damage[41]. Aslan *et al.*[42] reported that EA treatment increased the levels of caspase-3 and Nrf2 expression while simultaneously decreasing the levels of Bcl-2 and NF-kB expression. In the current study, Gu *et al.*[43] demonstrated that ellagic acid at the doses of 5 to 20 mg/kg had a protective effect on lipopolysaccharide (LPS)/D-galactosamine (GalN)-induced acute hepatic injury in mice. The pretreatment with EA was found to inhibit the phosphorylation of IκB-α and NF-κB p65. The results showed that EA protected against LPS/GalN-induced acute hepatic injury through the activation of Nrf2 and the inhibition of NF-κB activation.

3.2.4 Caffeic acid (CA). CA, also known as 3,4-dihydroxycinnamic acid, is a phenolic compound found in various vegetables, fruits, and herbs. Several studies have documented its significant therapeutic impact, demonstrating its effectiveness as a hepatoprotective, neuroprotective, and anti-diabetic agent[44].

Moreover, several reports have shown that CA prevented liver reperfusion injury, griseofulvin-, nickel-, or doxorubicin-induced hepatotoxicity, as well as paracetamol and CCl_4-induced liver damage[45-46].

Acetaminophen (APAP) is commonly utilized in clinical practice as an analgesic and antipyretic agent. However, an overdose of APAP can lead to significant liver injury[47]. The study by Pang *et al.* investigated the protective mechanism of CA in APAP-induced liver injury. The Kelch-like ECH-associated protein 1 (Keap1)-Nrf2 signaling pathway is thought to play a critical role in liver oxidative injury and has been identified as a potential therapeutic target for liver disease[48]. The protective effect of CA against APAP-induced liver injury is mediated through the downregulation of Keap1 expression, which inhibits the binding of Keap1 to Nrf2, and thus activating Nrf2 and leading to increased expression of antioxidative signals including HO-1 and NQO1[45].

3.2.5 Gallic acid (GA). GA, also known as 3,4,5-trihydroxybenzoic acid, belongs to a class of phenolic compounds and is classified as a natural secondary metabolite[49]. A prevalent pharmacological side effect encountered during the treatment of tuberculosis is drug-induced hepatotoxicity caused by anti-tuberculosis medications[50]. A study by Sanjay *et al.* [51] pointed out that gallic acid is effective in preventing hepatotoxicity induced by isoniazid and rifampicin. This effectiveness is attributed to the upregulation of endogenous antioxidant gene expression via the Nrf2 pathway, as well as the inhibition of NF-κB mediated proinflammatory signaling pathway.

Diclofenac (DIC) is classified as a nonsteroidal anti-inflammatory drug, and consumption of this drug creates side effects such as liver injury[52]. Studies have shown that GA exhibits a therapeutic effect on liver injuries induced by diclofenac (DIC)[53], fluoxetine (FLU)[54], paracetamol (PCT)[55] and aflatoxin B1[56]. GA has been shown to alleviate abnormal biochemical parameters and tissue pathological changes. GA increases the SOD and CAT activity in the liver while concurrently decreasing levels of liver MDA levels. GA improves liver injury by inhibiting inflammatory responses, reducing oxidative stress, and promoting apoptosis.

3.2.6 Chlorogenic acid (CGA). CGA, also known as 5-O-caffeoylquinic acid (5-CQA), has been identified as a potent polyphenolic antioxidant because it contains a certain amount of the R-OH group, which can bind with hydroxyl radicals and superoxide anion radicals to protect cells from oxidative injury[57]. CGA is associated with a variety of significant therapeutic effects, including antioxidant activity, antibacterial properties, hepatoprotective effects, anti-inflammatory actions, antipyretic effects, neuroprotection, anti-obesity effects, antiviral and antimicrobial activities, anti-hypertensive properties, free radical scavenging, and stimulation of the central nervous system[58].

Tamoxifen (TAM) is chemotherapeutic agent frequently employed in clinical settings for the treatment of advanced breast and ovarian cancer. However, its use is accompanied by considerable side effects, notably hepatotoxicity. The study conducted by Owumi *et al.* [59] demonstrated that CGA alleviated TAM-mediated toxic responses, restored antioxidant capacities, reduced oxidative stress, and lowered the levels of pro-inflammatory cytokines, as well as the activities of caspase-3 and caspase-9 in experimental rats.

4 Pharmacological roles of pomegranate and its bioactive components in nonalcoholic fatty liver disease (NAFLD)

NAFLD is a metabolic syndrome characterized by excessive fat deposition in hepatocytes. It is also associated with metabolic stress-induced liver injury, which is closely related to insulin resistance and genetic susceptibility[60].

4.1 Extracts Previous research has demonstrated the anti-obesity effects of pomegranate[8]. Ahmed *et al.* have shown that pomegranate consumption resulted in a reduction of body weight gain, food intake, and serum levels of lipids, leptin, and glucose in obese rats through the upregulation of hepatic mRNA expression of hormone-sensitive lipase, pyruvate kinase, and fatty acid synthase[61]. The administration of pomegranate extract resulted in a reduction of uncoupling protein 2 (UCP2) expression, restoration of ATP content, suppression of mitochondrial protein oxidation, and improvement of mitochondrial complex activity in the liver, as well as reduced expression of pro-inflammatory cytokines in hepatocytes[61]. Long-term consumption of PJ is indeed beneficial for health, particularly in improving serum glycation index and triglyceride levels, as well as downregulating pro-inflammatory and profibrotic gene expression in the liver, thereby playing a positive role in the prevention of NAFLD[62].

4.2 Monomer

4.2.1 Punicalagin. A study by Liu *et al.* points out that punicalagin exhibits protective effects against NAFLD. Compared to the Western diet-fed (WD) group, the mice treated with punicalagin have lower fat content in the liver. Punicalagin has been shown to modulate the transcriptional expression of key genes involved in the fatty acid oxidation pathway, thereby alleviating glucose intolerance[61]. Punicalagin has been shown to enhance adiponectin signaling and lipid metabolism within visceral adipose tissue. Furthermore, punicalagin improves gut microbiota dysbiosis induced by WD and enhances gut barrier function[61]. In conclusion, punicalagin protects against NAFLD via regulating lipid homeostasis, adipokine production, and oxidative stress levels. Additionally, it contributes to the restoration of microbiota balance and impaired gut barrier function[61, 63]. Zou *et al.* [61] discovered that punicalagin protected against high-fat diet (HFD)-induced NAFLD by increasing mitochondrial function and reducing oxidative stress and inflammation. Finally, the study demonstrated that punicalagin lowered triglyceride and cholesterol content in HepG2 cells and protected cells from palmitate induced mitochondrial dysfunction and insulin resistance.

4.2.2 CCA. CGA also has antitumor, antihypertensive and lipid-lowering functions. The study by Shi *et al.* [64] explores the impact of CGA on NAFLD induced by a high-fat diet (HFD). The study indicates that CGA could alleviate HFD-induced hepatic steatosis and inflammation, reduce serum transaminase, fasting

blood glucose (FBG), and blood lipids, while also increasing insulin sensitivity. Furthermore, the study reveals that CGA is capable of reversing the activation of the TLR4 signaling pathway in the liver. Moreover, the study suggests that CGA may confer protection against NAFLD, probably through its anti-inflammatory effects associated with the regulation of gut microbiota and an increase in glucagon-like peptide-1 (GLP-1) secretion.

4.2.3 CA. CA demonstrates the ability to reduce hepatic lipid accumulation, reverse the imbalance in the gut microbiota, and attenuate lipopolysaccharide-mediated inflammation[65]. Consequently, CA effectively inhibits the dysregulation of gene expression associated with lipid metabolism. Mu *et al.*[65] have demonstrated that CA exerts a protective effect against the development of HFD-induced NAFLD through integrative mechanisms, which include the inhibition of gut microbiota dysbiosis, the alleviation of endotoxemia and the reduction of proinflammatory reponse.

4.2.4 GA. Adenosine monophosphate-activated protein kinase (AMPK) plays a crucial role in disrupting the balance of redox status between lipogenesis and lipid oxidation[66]. When activated, AMPK phosphorylates and inactivates acetyl-CoA (ACC), thereby preventing the conversion to malonyl-CoA for fatty acid synthesis and subsequently inhibiting hepatic lipid accumulation. Furthermore, evidence suggests that AMPK can also activate peroxisome proliferation-activated receptor α (PPARα), a key regulator of mitochondrial fatty acid β-oxidation, which enhances fatty acid uptake[67]. On the one hand, GA reduces hepatic lipid accumulation and protects against hepatic lipotoxicity by activating the AMPK-ACC-PPARα signaling pathway[67]. On the other hand, GA attenuates the overproduction of hepatic mtROS via an overall improvement in mitochondrial functions[67].

According to the findings, the protective effects of pomegranate extract and its monomer components on NAFLD are primarily evident in several key areas: regulating lipogenesis-related genes, reducing fat accumulation, improving oxidative stress and antioxidant activity, reducing inflammation, improving liver function, regulating blood glucose levels, and lowering blood lipid levels.

5 Pharmacological roles of pomegranate and its active components in liver fibrosis

The central link in the development of liver fibrosis is the activation and proliferation of static hepatic stellate cells, leading to excessive deposition of collagen-dominated extracellular matrix (ECM). Studies show that liver fibrosis is a stress response to liver injury. Furthermore, chronic inflammation and oxidative stress have been identified as critical contributors to the development of hepatic fibrosis. Advanced liver fibrosis can lead to complications such as cirrhosis, liver failure, portal hypertension, and hepatocellular carcinoma[68]. At present, the primary mechanisms of anti-fibrosis drugs include the inhibition of liver inflammation and immune responses, the migration of oxidant injury, the suppression of HSC activation, the reduction of ECM synthesis and the acceleration of ECM degradation[69].

5.1 Extract Pomegranate peels and seeds have been verified to possess antioxidant activity, primarily due to their rich content of polyphenols, flavonoids, and structurally related compounds[70]. According to the research, pomegranate peels extract (PPE) and seeds extract (PSE) have preventive effects against liver fibrosis. The underlying mechanisms of action may be linked to their antioxidant activities, the reduction of TGF-β1 levels, and the inhibition of collagen formation. Furthermore, these extracts have been shown to reduce the CCl_4-induced increase in the levels of hydroxyproline (Hyp), hyaluronic acid (HA), laminin (LN), and procollagen type III (PC III)[71].

Similarly, research has demonstrated that PPE provides protection against hepatic fibrosis induced by biliary obstruction through its antioxidant, antifibrotic, and free radical scavenging properties. Treatment with PPE has been shown to improve bile duct ligation (BDL)-induced liver fibrosis and to reduce the BDL-induced increase in serum LDH activity, as well as plasma levels of TNF-α and IL-1β. Moreover, the elevated hepatic lipid peroxidation, MPO activity, and collagen content, as well as the reduced GSH levels observed following BDL, were effectively reversed by PPE treatment[72].

The supplementation of pomegranate juice (PJ) exhibits an antifibrotic effect by upregulating Nrf2 and modulating the crosstalk between transcription factors Nrf2 and NFκB. This finding demonstrates a potential mechanism underlying its therapeutic action in the treatment of fibrosis[73].

Studies have shown that pomegranate extract (PE) can alleviate thioacetamide (TAA)-induced liver fibrosis in rats by downregulating the NF-kB and TGF-β/Smad3 signaling pathways[74]. Also, PE has been shown to reduce the expression of inflammatory biomarkers in the liver. The extract effectively inhibits the TGF-β/Smad3 signaling pathway, resulting in the normalization of both phospho-Smad3 protein expression and collagen-1 expression. The results show that treatment with PE inhibits oxidative stress, inflammation, and fibrogenesis by promoting the Nrf2/HO-1 pathway while concurrently inhibiting the NF-κB/TGF-β/Smad3 pathways[75].

5.2 Monomer

5.2.1 CGA. The study showed that CGA has an anti-liver fibrosis effect on CCl_4-induced mice through its interaction with the miR-21-regulated TGF-β1/Smad7 signaling pathway[76]. In addition, CGA may prevent liver fibrosis by activating the Nrf2 pathway and inhibiting the NOX/ROS/MAPK pathway. *In vivo* investigations revealed that CGA significantly reduced the degree of liver fibrosis, and the expression of α-SMA, Collagen I, Collagen III, and TIMP-1 in CCl_4-injected rats. CGA treatment reduced the expression of CYP2E1 and inhibited the phosphorylation of p38 and ERK1/2[77].

5.2.2 GA. In a previous study, GA exhibited an antifibrotic effect in cultured HSC-T6 cells. The antifibrotic mechanism of GA involves the modulation of gene expression and protein levels of the Bcl-2/Bax family, which subsequently induces HSC-T6 cell apoptosis[78]. In addition, GA can effectively reduce the accumulation of extracellular matrix[79]. A study by Chen *et al.*[80] points out that GA can reduce dimethylnitrosamine (DMN)-induced liver fibrosis in rats by relying on its superior antioxidant capacity and

taking part in the regulation of cytokine expression. GA demonstrates the potential to repair liver fibrosis, reduce liver damage, and boost antioxidant capacity in liver tissues. Previous studies have shown that GA exhibits hepatoprotective and antioxidant effects against TAA-induced liver fibrosis. The study showed that the administration of GA led to a reduction of miR-21 expression while simultaneously upregulating the expressions of miR-30 and miR-200 expressions as a consequence of their inhibition of the TGF-β1/Smad3 signaling pathway[81].

In conclusion, a substantial body of research has indicated that pomegranate extract and its active components can prevent HSC activation and collagen deposition, thereby mitigating liver fibrosis. CGA and GA have been shown to restrict HSC proliferation via multiple signal pathways while simultaneously reducing inflammatory responses, thereby reducing liver fibrosis. This provides useful inspiration and research direction for the integrated mechanism of pomegranate against liver fibrosis.

6 Pharmacological roles of pomegranate and its active components in hepatitis

6.1 Extract Autoimmune hepatitis is a relatively rare idiopathic syndrome characterized by the immune-mediated destruction of hepatocytes. Concanavalin A (Con A)-induced hepatitis is a form of immune hepatitis that arises from T-cell-mediated cytokine imbalances, which can resemble the pathological features observed in various viral and autoimmune hepatiti conditions[82]. The study conducted by Wang *et al.*[83] elucidated the impact of PPE on ConA-induced autoimmune hepatitis. The results indicated that pretreatment with PPE significantly alleviated inflammatory infiltration. Flow cytometry analysis demonstrated a reduction in the immune response within the liver under PPE-pretreated conditions. Furthermore, pretreatment with PPE reduced the infiltration of activated CD_4 and CD_8 T cells in the liver.

6.2 Monomer

6.2.1 Punicalagin and punicalin. Hepatitis C virus (HCV) is linked to the deregulation of both innate and adaptive immune signaling pathways, in addition to virus-induced cytotoxicity[84]. The methanolic extract of pomegranate peel and juice showed inhibitory effects on HCV NS3/4A protease activity *in vitro*. Further, punicalagin and punicalin significantly reduced HCV replication in the cell culture system[85]. Moreover, both punicalagin and punicalin resulted in a considerable reduction of HCV RNA levels in HCV subgenomic replicon and infectious cell culture systems using the HCV-JFH1 (genotype-2a) virus and H77S (genotype-1a) RNA[85].

6.2.2 EA. Jae *et al.* have demonstrated that EA mitigates Con A-induced hepatitis. The underlying mechanism of this observed effect involves the inhibition of TLR2 and TLR4 expression, which leads to a decrease in the expression of inflammatory cytokines. This process consequently inhibits the phosphorylation of MAPK and the nuclear translocation of NF-κB. Further, pretreatment with EA decreases the expression of pro-inflammatory cytokines, such as TNF-α, IL-6, and IL-1β, and diminishes the phosphorylation of JNK, ERK1/2, and p38[86].

6.2.3 CGA. CGA inhibits the NF-κB pathway in chronic restraint stress-induced liver inflammation by upregulating resolvin D1. The research conducted by Wang *et al.*[87] demonstrates that CGA has the ability to inhibit liver inflammation associated with chronic stress through the upregulation of Resolvin D1 (RvD1). The findings indicate that CGA significantly reduces hepatic hemorrhage and inflammatory cell infiltration, alleviates hepatic injury, inhibits the activation of the NF-κB pathway, and decreases the expression levels of IL-1β, IL-6, and TNF-α in the liver[87]. RvD1 is a derivative of polyunsaturated fatty acids that has inhibitory effects on a variety of inflammatory diseases. Furthermore, CGA has been shown to increase RvD1 levels in both serum and liver. In conclusion, CGA upregulates the production of RvD1, inhibits the expression of IRAK1 and TRAF6 upstream of NF-κB, and prevents p65 from entering the cell nucleus. This cascade of events ultimately reduces the mRNA transcription of inflammatory factors and ameliorates liver injury, indicating that CGA has a protective effect on chronic stress-induced inflammation[87].

7 Pharmacological roles of pomegranate and its active components in liver cancer

Liver cancer, specifically hepatocellular carcinoma (HCC), is one of the most prevalent malignancies, followed by intrahepatic cholangiocarcinoma[88-89]. As a primary liver cancer, HCC is the sixth most commonly diagnosed cancer and represents the second leading cause of cancer-related mortality globally[90]. Researchers have found that pomegranate and its active components can effectively influence a variety of signaling pathways involved in inflammation, cellular transformation, hyperproliferation, angiogenesis, tumorigenesis initiation, and ultimately inhibit tumorigenesis and metastasis[91]. Pomegranate and its active components exhibit significant anticancer activity in various cancer cells, such as bladder, skin, breast, prostate, lung, leukemia, and colon cancers[91-92].

7.1 Extracts

7.1.1 Peel polyphenols. Song *et al.*[93] evaluated the effect of pomegranate peel polyphenols (PPPs) on the proliferation and apoptosis of HepG2 cells. The findings demonstrated that PPPs at the concentrations of 100, 200, and 300 μg/mL effectively halted the HepG2 cell cycle at the S-phase and that the amount of early apoptotic cells and ROS levels were obviously enhanced in a dose-dependent way. It could be concluded that PPPs could significantly inhibit the proliferation of HepG2 cells, induce apoptosis, and arrest the cell cycle in these cells through the regulation of the intrinsic mitochondrial pathway[93-94].

7.1.2 Emulsion. Pomegranate emulsion is a proprietary combination of pomegranate aqueous phase extract and pomegranate seed oil. Bishayee *et al.*[94] discovered that a pomegranate emulsion had chemo-preventive properties against diethylnitrosamine (DENA)-induced hepatocarcinogenesis in rats. Moreover, the application of pomegranate emulsion reduced the number, multiplicity, size, and volume of hepatic nodules. In addition, pomegranate emulsion increased protein and messenger RNA expression of the hepatic Nrf2[94].

In a separate study, Bhatia *et al.* demonstrated that pomegranate emulsion exerted inhibitory effects on hepatic cancer through antiproliferative and proapoptotic mechanisms by modulating the Wnt/β-catenin signaling pathway. This is significant because NF-κB can stimulate the Wnt/β-catenin signaling pathway, which is implicated in cell proliferation, cell survival, and apoptosis evasion. Besides, pomegranate emulsion reduced the proliferation of liver (proliferating cell nuclear antigen) and change in cell cycle progression (cyclin D1) following DENA-induced hepatocarcinogenesis in rats. Additionally, pomegranate emulsion dose also reduced hepatic β-catenin and augmented glycogen synthase kinase-3β expression. Furthermore, pomegranate emulsion upregulated the proapoptotic protein Bax while downregulating the antiapoptotic protein Bcl-2, thus inducing intrinsic apoptosis and facilitating the elimination of transformed cells during hepatocellular carcinogenesis[95]. The results of these studies emphasize that pomegranate emulsion ameliorates HCC by modulating the Nrf2, NF-κB, and Wnt/ β-catenin signaling pathways.

7.1.3 Juice extract. Another study by Aya *et al.* established a model of DENA-induced hepatocarcinogenesis in male albino rats to clarify the role of PJ in the prevention of HCC development. The findings indicated that PJ effectively inhibited hepatic nodule formation and suppressed nodule growth. Oral administration of PJ decreased DENA-induced HCC via improvement of functional and histopathological parameters. Furthermore, PJ induced the downregulation of miR-21, promoted the upregulation of caspase-3 and Bax mRNA expressions, and triggered apoptosis. Moreover, PJ was effective in ameliorating the hepatic antioxidant defense state and the inflammatory status[96].

7.2 Monomer A study by Chung *et al.*[97] confirmed that CA suppressed the growth of HepG2 tumor xenografts in nude mice *in vivo*. Other experiments have shown that CA, which is a strong and selective inhibitor of matrix metalloproteinase (MMP)-9 activity and transcription, can also induce apoptosis in cancer cells by enhancing ROS levels and impairing mitochondrial function. Consequently, studies have shown that CA has protective effects against liver cancer, which are mediated through the selective suppression of MMP-9 enzyme activity and transcriptional down-regulation via the dual inhibition of NF-κB and MMP-9 catalytic activity.

8 Conclusions and future opportunities

In this review, we systematically summarize the research findings regarding the hepatoprotective effects of pomegranate extract and its active components, while also discussing their potential mechanisms of action. Pomegranate extract and its active components have demonstrated significant potential in the treatment of various liver diseases.

As shown in Fig. 1, the mechanisms by which pomegranate extract and its active components protect the liver may involve multiple aspects, including antioxidation, anti-inflammation, antifibrosis, and anti-apoptosis. Pomegranate can reduce the activity of liver injury markers in serum, such as ALT, AST, and TBIL, thereby improving liver function. For example, pomegranates can regulate the fat-generating genes in the liver, reducing fat accumulation in the liver, and have a positive preventive and therapeutic effect on NAFLD. Pomegranate can inhibit the activation and proliferation of hepatic stellate cells, reducing the occurrence of liver fibrosis. Pomegranate can reduce liver inflammation responses, such as lowering the levels of pro-inflammatory cytokines TNF-α and IL-6, and increasing the levels of the anti-inflammatory cytokine IL-10. Pomegranate can also upregulate the anti-apoptotic factor Bcl-2 and downregulate pro-apoptotic factors such as Bax and caspase-3, thereby inhibiting the process of apoptosis. At the molecular level, pomegranate reduces oxidative stress and protects liver cells from damage by activating antioxidant signaling pathways such as MAPK, Keap1/Nrf2, and Nrf2/HO-1. Pomegranate alleviates liver inflammation by regulating inflammatory signaling pathways such as NF-κB and TGF-β/Smad. Pomegranate exerts antiapoptotic and autophagy-regulating effects by modulating the P62/SQSTM1 and AKT/FOXO3a pathways. Pomegranate can inhibit the proliferation of HepG2 cells, induce apoptosis, and block the cell cycle. Additionally, it can suppress the formation and growth of liver nodules by regulating the Wnt/β-catenin signaling pathway. Due to the association between gut microbiota imbalance and the development of various liver diseases, pomegranates may also reverse gut microbiota imbalance. Pomegranates demonstrate superiority in liver disease treatment through multi-component, multi-link, and multi-pathway interventions. This multi-target mechanism of action provides new insights for treating complex diseases.

Pomegranate, as a plant that is both a food and a medicine, necessitates comprehensive safety research to facilitate its application in dietary supplements and clinical applications. According to existing research data, pomegranate extract and its active components have shown good safety. Preliminary studies have revealed that hydroalcoholic extract and ethanolic extracts of the whole pomegranate fruit demonstrate a good safety profile with an acute LD_{50} value of 731.1 mg/kg · bw in the tested animals[98]. A study by Patel *et al.*[99] pointed out that the acute and subacute toxicity studies of pomegranate fruit extracts at high dosage (600 mg/kg · bw) did not cause any adverse effects and had no observed adverse effect level in rats. Pomegranate seed oil, which is a component of pomegranate extract, has also been studied for its safety. Moreover, the no observable effect level for pomegranate seed oil was estimated at 50 000 ppm (4.3 g of pomegranate seed oil per kg body weight per day)[100].

Pomegranate, as a natural product, has indeed received widespread attention for its monomer components and extracts in the prevention and treatment of liver diseases. The components of pomegranate have been confirmed to possess antioxidant, anti-inflammatory, antifibrotic, anti-lipotoxic, anti-hepatocellular carcinoma, and hepatoprotective properties. Moreover, the positive effects of pomegranate extract on the gut microbiota also indicate its potential role in regulating metabolism and preventing obesity and related metabolic diseases. In summary, the application prospects of pomegranate extract in the treatment of liver diseases are broad, but more clinical research is needed to support its widespread clinical use. We hope this review can provide some insights

for the further development of natural drugs for treating liver diseases. Perhaps in the future, pomegranates will be more widely used as dietary supplements, in combination with other medications, to treat liver diseases.

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