Protective Effects of *Morus nigra* and Its Phytochemicals against Hepatotoxicity: A Review of Preclinical Studies

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Keywords

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Abstract

Background: Our liver has a variety of vital functions including removing poisons, storing energy, immunological roles, and secretory and excretory functions. It may face some kinds of diseases caused by viruses, hepatotoxic chemicals, drugs, alcohol, and inherited disorders. Oxidative stress and inflammation are in the core of mechanisms of liver damages induced by viruses or chemical agents. **Summary:** *Morus nigra* (*M. nigra*), generally known as black mulberry, exhibited wide-spectrum pharmacological effects including antidiabetic, antinociceptive, anticancer, and hepatoprotective activities. Different parts of this plant particularly the fruit and leaf have shown beneficial effects on hepatocytes in cell culture and animal models of liver damages induced by chemicals (e.g., CCl₄), drugs (e.g., paracetamol), diet (e.g., high fat), diabetes, etc. The beneficial effects of *M. nigra* on the liver are attributed to the presence of considerable amounts of phenolic compounds such as anthocyanins, flavonols, and phenolic acids. The present review is aimed to focus on the hepatoprotective activities of *M. nigra* and its

phytochemicals and the mechanisms responsible for these activities. **Key Messages:** The evidence reviewed in this study can help design clinical trials on *M. nigra* in patients with liver disorders and develop a hepatoprotective herbal medicine.

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Introduction

The liver, as the largest organ inside our body, has a variety of vital functions including removing poisons, storing energy, immunological roles, and secretory and excretory functions [1]. It may face some kinds of diseases caused by viruses (e.g., hepatitis A, B, and C), hepatotoxic chemicals, drugs, too much alcohol (e.g., fatty liver disease and cirrhosis), and inherited disorders (e.g., Wilson disease and hemochromatosis). Although there are some symptoms like yellowing of the skin and eyes as well as swelling of the abdomen and legs, sometimes no symptoms are observed in the patients [2].

In the last decade, considerable progress has been made in our understanding of the pathophysiology of liver disorders. Oxidative stress and inflammation are in the

Fig. 1. The main phenolic compounds found in *Morus nigra*.

core of mechanisms of liver damages induced by viruses or chemical agents [3, 4]. Antioxidant and anti-inflammatory effects of medicinal plants and their phytochemicals have been well documented [5, 6]. Therefore, finding effective and safe natural hepatoprotective agents is one of the future directions.

Berries, particularly the popular ones such as blueberry, strawberry, raspberry, and blackberry, are among the best plants with medicinal value due to the presence of a wide range of bioactive phenolic components [7]. These plants exhibited wide-spectrum biological effects including antidiabetic, anticancer, antiaging, neuroprotective, and lipid metabolism regulatory activities [7–10]. Blackberry (black mulberry) with the scientific name of *Morus nigra* (*M. nigra*) grows in different parts of the world par-

ticularly in Southwestern Asia and Europe. The fruits and leaves of this plant have shown remarkable antioxidant, anti-inflammatory, and antiviral activities [11–14]. The present review is aimed to focus on the hepatoprotective effects of *M. nigra* and its phytochemicals.

Phytochemical Profile of *M. nigra*

There are about 4,000 different phytochemicals found to be responsible for taste, color, and other related functions of plants [15]. Two main forms of plant-derived phytochemicals include primary types (e.g., amino acids, carbohydrate, proteins, and chlorophyll) and secondary phytochemicals (e.g., flavonoids, terpenoids, al-

kaloids, saponins, anthraquinones, and volatile oils) [16]. The main phytochemical compounds found in *M. nigra* are shown in Figure 1. The leaf and fruit contain considerable amounts of phenolic compounds such as anthocyanins (e.g., cyanidin-3-glucoside, cyanidin-3-rutinoside, and pelargonidin), flavonols (e.g., quercetin, rutin, and kaempferol), flavanones (e.g., naringenin), and phenolic acids (e.g., chlorogenic acid, gallic acid, and caffeic acid).

It has been well documented that phytochemical compositions of mulberries, like other plants, are influenced by the type of species, genetic differences, and geographical and environmental conditions. For example, *M. nigra* has higher concentrations of flavonoids compared to *Morus alba* (*M. alba*), the white mulberry [17–21]. Also, the levels of chlorogenic acid, syringic acid, O-coumaric acid, and vanillic acid were shown to be higher than those of *M. alba* and *Morus rubra* (red mulberry) [17]. The differences in the level of flavonoids justify the greater antioxidant and anticancer activities of *M. nigra* compared to *M. alba* and *Morus rubra* [17, 22, 23]. Also, a comparison of polyphenol composition and antioxidant activity of fresh *M. nigra* with different mulberry products (dried mulberry, mulberry wine, molasses, ice cream, juice, jam, and syrup) showed that fresh *M. nigra* has higher contents of total anthocyanins, phenolics, flavonoids, and antioxidant capacity [24]. For example, the content of anthocyanins in juice prepared by centrifugation, jam, jelly, juice prepared by thermal processing, syrup, canned fruit, and wine is approximately 106, 94, 90, 74, 47, 24, and 21 mg/dm³, respectively [25]. It is well known that the phytochemical profile reported for a given plant depends on the type of the extraction method used for the study. A comparative study on 3 different extracts (infusion, decoction, and methanolic) of *M. nigra* showed more antioxidant phenolic compounds for the infusion extract compared to the 2 other forms [26]. Evaluating the profile of the phenolic compounds of *M. nigra* fruit before and after fermentation showed that the largest level of flavonoids is extracted from fresh fruits than from fermented fruits [27]. Also, the acetonic and methanolic extracts of *M. nigra* and *M. alba* fruits were analyzed for their phenolic compounds, and results showed that acetonic and methanolic extracts of *M. nigra* have the highest contents of total phenolics (173 mg/g and 164 mg/g, respectively) [28]. Finally, genotypes strongly influence the chemical content and antioxidant capacity of *M. nigra* [23, 29]. Comparing the levels of organic acids, sugars, vitamin C, and phenolic compounds in 3 genotypes of this plant showed that there is a significant difference between the genotypes regarding the amounts of these compounds [23].

Pharmacokinetics and Biodistribution of *M. nigra* Phytochemicals

Contrary to in vitro conditions, the in vivo efficacy of phytochemicals mainly depends on their absorption, distribution, metabolism, and excretion. Pharmacokinetic studies have shown that flavonoids, the main phytochemical compounds found in *M. nigra*, can be absorbed from the gastrointestinal tract after oral administration [30–33]. Measurement of the levels of 6 flavonols (rutin, isoquercitrin, astragalin, quercetin, kaempferol, and isorhamnetin) in rat plasma following a single oral dose (1 g/kg) of mulberry leaves extract showed a maximum concentration ranging from 6.8 ± 4.6 to 132.5 ± 73.3 ng/mL, the time to reach the maximum plasma concentration of 0.19 ± 0.06 to 0.30 ± 0.13 h, and half-life of elimination ranging from 0.87 to 1.19 h [30]. The flavonoid glycosides (isoquercitrin and rutin) are released as aglycone after being hydrolyzed in the intestinal tract by bacteria [31]. Following absorption, flavonoids undergo first-pass phase II metabolism in the epithelial cells of the small intestine and the liver. The predominant forms of metabolites present in plasma are those conjugated with methyl, glucuronate, and sulfate groups [32]. Finally, they are excreted through the bile and the kidney [34]. A bio-distribution study of ¹²⁵I-labeled rutin and ¹²³I-labeled hesperetin in mice showed that in the first hour after oral administration, these flavonoids were found in the stomach and small intestines. Then, the observed radioactivity of the upper gastrointestinal tract was decreased while that of the large intestine displayed its maximum uptake after 3–4 h of administration. The labeled rutin and hesperetin were detected in different organs including the liver, lung, kidneys, heart, and spleen within 1–24 h [33, 34]. In addition to the animal studies described above, several clinical studies have addressed the pharmacokinetics of flavonoids in healthy volunteers. The results show considerable differences among the different types of flavonoids regarding absorption from the small intestine (ranging from 0 to 60% of the administered dose) and half-life (ranging from 2 to 28 h) [32].

Anthocyanins can be detected in the blood within minutes of oral consumption, suggesting that these compounds may be quickly absorbed from the stomach in addition to the small intestine. The extent of absorption of anthocyanins from the gastrointestinal tract is affected by their sugar moiety and acylated groups [35]. The exact mechanism behind the absorption of anthocyanins from the gastrointestinal tract is still uncertain. It has been suggested that bilitranslocase, an organic anion membrane carrier in the liver

Fig. 2. Potential mechanisms of hepatoprotective effects of *Morus nigra* and its phytochemicals. Inhibitory effects are shown by red lines and dotted arrows. cFLIP, cellular FLICE inhibitory protein; ER, endoplasmic reticulum; FFA, free fatty acids; FasL, Fas ligand; FOXO, forkhead box class O; HBV, hepatitis B virus; HCV, hepatitis C virus; JNK, c-Jun N-terminal kinase; ROS/RNS, reactive oxygen/nitrogen species; TNFR1, tumor necrosis factor receptor-1; TNF-α, tumor necrosis factor-alpha; TRAIL, TNF-related apoptosis-inducing ligand; TRAILR, TRAIL receptor.

and stomach, is involved in the bioavailability of anthocyanins [36]. Maximum plasma concentrations of total anthocyanins have been reported to be in the range of 1–100 nmol/L in human studies [35]. Marques et al. [37] analyzed the plasma of healthy participants following consumption of blackberry purees and observed that some anthocyanins (cyanidin-3-glucoside and cyanidin-3-rutinoside) could be absorbed intact as native compounds and extensively metabolized into methylated, glucuronidated, and sulfated derivatives. The maximum plasma concentrations of cyanidin-3-glucoside and cyanidin-3-rutinoside were 47 ± 8 ng/mL ($t_{\max}$: 66 ± 15 min) and 11 ± 3 ng/mL ($t_{\max}$: 108 ± 12 min), respectively. Also, they reported that methyl-cyanidin-glucuronide and 3-methyl-cyanidin-3-glucoside were the main anthocyanin conjugates found in the plasma. Bio-transformation of anthocyanins is done by enzymes located in the small intestine, liver, and kidney. Biodistribution

studies in rats and pigs showed that anthocyanins could be found in different organs such as the liver, eye, and even the brain [35]. Excretion of anthocyanins in human urine peaked between 2 and 4 h following consumption of anthocyanin-rich juice [38].

Molecular Mechanisms of Liver Damage

The strategic anatomical location, specific blood flow, and unique role in metabolism and detoxification of poisons render the liver particularly vulnerable to damage by chemicals, viruses, and metabolic disorders [39]. The pathogenesis of chemical-induced hepatotoxicity is mainly initiated by conversion into reactive intermediate species, such as reactive oxygen species (ROS) and reactive nitrogen species (Fig. 2). When the antioxidant ca-

Table 1. Effects of *M. nigra* extracts on the liver

n	Type of extract	Animal model	Method and duration of administration	Observed effects	Ref.
1	Fresh fruit	CCl ₄ -induced hepatotoxicity in rats	Diet supplemented with the fruit (30% w/w) for 10 days	↓AST and ALT ↓Urea ↓Lipid peroxidation ↓Fucosidase	[48]
2	Lyophilized extract of the fruit	CCl ₄ -induced hepatotoxicity in rats	Injection (150–300 mg/kg/day, i.p.) for 8 weeks	↓AST and ALT ↓GGT ↑SOD and GPx	[47]
3	Ethanollic extract of the leaf	Methotrexate-induced hepatotoxicity in rat	Oral (500 mg/kg/day) for 14 days	↓AST, ALT, ALP, and lactate dehydrogenase	[54]
4	n-Hexane extract of the leaf	Paracetamol-induced hepatotoxicity in mice	Oral (250 and 500 mg/kg) for 7 days	↓AST, ALT, and ALP ↓Total bilirubin Histopathological improvements of hepatocytes	[51]
5	Hydroalcoholic extract of the leaf	Alloxan-induced diabetes in rats	Oral (500 mg/kg/day) for 30 days	↓AST and ALT ↓SOD/catalase ratio ↓Hyperglycemia	[60]
6	Hydroalcoholic extract of the leaf	Paracetamol-induced hepatotoxicity in mice	Oral (250 and 500 mg/kg/day) for 7 days	↓AST, ALT, and ALP ↓Total bilirubin	[52]
7	Aqueous extract of the leaf	Streptozotocin-induced diabetes in mice	Oral (100 mg/kg) twice daily for 42 days	↑AST and ALT ↓Hyperglycemia	[79]
8	Ethanollic extract of the leaf	Acute and subacute toxicity studies in rats	Oral (500, 750, and 1,000 mg/kg/day) for 28 days	↓AST and ALT ↓Total cholesterol ↑Hepatic and renal lipid peroxidation	[80]
9	Ethanollic extract of the fruit	High fat diet-fed rat	Oral (100 and 200 mg/kg/day) for 10 weeks	↓AST and ALT ↓Hepatic triglycerides	[57]
10	Hydroethanollic extract of the pulp and leaf	Lipopolysaccharide-induced sepsis in mice	Oral (500 mg/kg/day) for 21 days	↓Inflammatory cells in the liver ↑Animal's survival ↓TNF level ↔ IL-1β, IL-6, and IL-10 ↓Glutathione peroxidase activity	[63]
11	Ethanollic extract of the leaf	Streptozotocin-induced diabetes in rat	Oral (up to 13.5 g/kg/day) for 4 weeks	↓AST and ALT ↓Urea and creatinine ↓α-Glucosidase activity	[58]
12	Methanollic extract of the leaf	D-Galactose-induced aging in mice	Oral (50–100 mg/kg/day) for 8 weeks	↓Lipid peroxidation in the liver ↑SOD, GPx, and catalase	[62]
13	Ethanollic extract of the leaf	Alloxan-induced diabetes in rat	Oral (200–400 mg/kg/day) for 14 days	↓Hyperglycemia ↓Dyslipidemia ↓Lipid peroxidation ↑Glutathione	[59]
14	Hydroethanollic extract of the leaf	Streptozotocin-induced diabetes in rat	Oral (250 mg/kg/day) for 4 weeks	↑Serum albumin ↓Urea, creatinine, and uric acid	[61]

Table 1 (continued)

n	Type of extract	Animal model	Method and duration of administration	Observed effects	Ref.
15	Fresh fruits	CCl ₄ -induced damage in HepG2 cells	Incubation with 0.01–1 mg/mL	↓AST and ALT ↑GSH and SOD	[49]
16	Hydroethanolic and trifluoroacetic acid extracts of fresh fruit	High-fat diet-induced hepatic steatosis in mice	Oral (100 mg/kg/day) for 14 weeks	↓AST and ALT ↓Hepatic steatosis and insulin resistance ↓Liver cholesterol and triglycerides	[55]
17	Ethanol extract of the fruit	High-fat diet-induced atherosclerosis in rats	Oral (105–210 mg/kg/day) for 6 weeks	↓Serum lipids ↓Atherogenic index ↓Lipid peroxidation in the liver ↑SOD, GPx, and catalase	[56]

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; GPx, glutathione peroxidase; GSH, glutathione; SOD, superoxide dismutase; ↓, decrease; ↑, increase; ↔, no significant effect.

capacity of hepatocytes is overwhelmed by the excess generation of these species, oxidative stress occurs, which in turn deregulates cellular signaling pathways, alters the function and structure of biomolecules, and eventually promotes cell death through apoptosis, autophagy, necrosis, pyroptosis, and ferroptosis processes [39–42]. In the case of metabolic disorders, for example, nonalcoholic fatty liver disease (NAFLD), accumulation of fatty acids leads to the formation of lipotoxic species in hepatocytes that causes endoplasmic reticulum stress and excessive level of ROS and proinflammatory cytokines [43]. Inflammatory cytokines (e.g., TNF- α , IL-1 β , and IL-18) can activate nuclear factor-kappa B and c-Jun N-terminal kinases pathways, which in turn upregulate the expression of these cytokines and therefore worsen inflammation, insulin resistance, and fibrosis. Activation of toll-like receptor family members in hepatitis can mediate steatosis, insulin resistance, and fibrosis in the liver [43, 44]. In NAFLD, the increase in mitochondrial fatty acid oxidation and stimulation of the tricarboxylic acid cycle enhance the delivery of reducing equivalents to the electron transport chain. This excites the formation of superoxide as a precursor of ROS. Overproduction of ROS can damage mitochondria through degradation of mitochondrial structural integrity, interruption of electron transport chain, and permeabilization of the outer mitochondrial membrane. The antioxidant system can inhibit the vicious cycle of mitochondrial dysfunction. However, in hepatic disorders, parallel to the increased level of ROS, the reduced activity of the antioxidant system (e.g., glutathione, catalase, and superoxide dismutase [SOD]) can promote hepatocyte damage [45, 46].

Effects of *M. nigra* Extracts on Hepatic Disorders

Throughout human life, various hepatotoxic chemicals and drugs can enter the body and affect the liver. Different parts of *M. nigra* particularly the fruit and leaf showed a beneficial effect on hepatocytes in cell culture and animal models of liver damages induced by chemicals (CCl₄), drugs (paracetamol and methotrexate), diet (high fat), diabetes, etc. (Table 1).

The CCl₄ has been shown to increase serum levels of liver enzymes, increase oxidative stress in hepatocytes, and induce pathological changes (increased numbers of Kupffer cells, vacuolization, and pericentral vein necrosis) in the liver of rats exposed to this compound [47]. Administration of the *M. nigra* extract to the CCl₄-treated cells/animals could significantly increase the activities of

Table 2. Effects of *M. nigra* phytochemicals on the liver

Constituent	Animal model	Method and duration of administration	Observed effects	Ref.
Cyanidin-3-glucoside	Alcohol-induced liver fibrosis in mice	Oral (200 mg/kg/day) for 12 weeks	↓Liver fibrosis ↓Stellate cells activity ↓AST and ALT ↓Liver total cholesterol and triglycerides ↑SOD ↓Lipid peroxidation	[69]
	CCl ₄ -induced hepatotoxicity in mice	Oral (10 mg/kg/day) for 5 days	↓AST and ALT ↓Lipid peroxidation ↓Histopathological changes ↑SOD, catalase, GPx, and glutathione reductase ↓IL-6	[70]
	Apolipoprotein E-deficient mice	Diet supplemented with C3G (0.06% w/w) for 12 weeks	↓Liver total cholesterol ↓Serum lipids ↓Atherosclerotic plaque formation ↑Bile acid synthesis	[71]
	High-fat diet-induced metabolic syndrome in rats	Oral (8 mg/kg/day) for 16 weeks	↓Bodyweight gain ↓Abdominal fat pads ↓Serum lipids Improving hepatic structure	[68]
Delphinidin	High-fat/high-carbohydrate diet-induced obesity in mice and palmitate-induced lipid accumulation in HepG2 cells	Oral (15 mg/kg/day) for 4 weeks	↓Triglyceride accumulation in HepG2 cells ↔Bodyweight, blood glucose, and liver histological abnormalities	[74]
	CCl ₄ -induced hepatotoxicity in mice	Injection (10–25 mg/kg/day, i.p.) for 2 weeks	Reversion of liver fibrosis Inactivation of HSC ↓AST, ALT, and ALP ↓TNF-α and TGF-β expression ↑GPx ↑Reduced/oxidized GSH	[73]
Pelargonidin	Lipopolysaccharide-induced hepatic failure in mice	Injection (0.2, 0.4, or 0.6 mg/kg, i.v.) 12 h after lipopolysaccharide injection	↓Mortality ↓AST and ALT ↓TNF-α, IL-6, and IFN-β	[75]
	Human hepatocytes	Incubation with 50 μM of pelargonidin for 48 h	↓Cytochromes P450 1A catalytic activity	[77]
	Citrinin-induced oxidative stress in HepG2 cells	Pretreatment with 50–100 μM of pelargonidin for 2 h	↑Cell viability ↑SOD and catalase ↑GPx and total GSH	[76]

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GPx, glutathione peroxidase; GSH, glutathione; SOD, superoxide dismutase; ↓, decrease; ↑, increase; ↔, no significant effect.

glutathione peroxidase and SOD [47–49]. The extract also decreased lipid peroxidation, prevented an increase in the levels of aspartate aminotransferase (AST) and gamma-glutamyl transferase, and improved the histological appearance of the liver [47, 48].

Overdose of paracetamol leads to accumulation of its metabolite N-acetyl-p-benzoquinone imine, which covalently binds to proteins following depletion of glutathione in hepatocytes. The loss of glutathione increases the formation of ROS and nitrogen species and therefore leads to mitochondrial dysfunction, lipid peroxidation, and even acute hepatic necrosis [50, 51]. Hepatoprotective activity of hydroalcoholic and n-hexane extracts of *M. nigra* leaves has been evaluated in paracetamol-intoxicated mice. The extracts at doses of 250 mg/kg and 500 mg/kg could reduce the levels of liver enzymes and total bilirubin and improve the histology of the liver. These beneficial effects of *M. nigra* were comparable to those of silymarin, a well-known hepatoprotective phytochemical [51, 52].

Methotrexate, an immune system suppressant and chemotherapy agent, is another drug with potential hepatotoxic side effects (e.g., increased risk of elevated transaminase levels) [53]. The ethanolic extract of *M. nigra* leaves was shown to decrease the serum levels of AST, alanine aminotransferase (ALT), alkaline phosphatase, and lactate dehydrogenase in rats with methotrexate-induced liver injury [54].

In studies on high-fat diet-fed animals, *M. nigra* was shown to improve hepatic steatosis, decrease serum lipids (cholesterol, triglyceride, and low-density lipoprotein), decrease hepatic lipid peroxidation, enhance antioxidant enzyme activities, alleviate insulin resistance, and improve glucose homeostasis. These effects were associated with suppressed expression of glycerol kinase and fatty acid desaturase 2 and enhanced expression of adiponectin receptor-2 and insulin-induced gene-1 [55, 56]. In another study on high-fat diet-induced NAFLD in rats, administration of mulberry fruit extract for 10 weeks decreased the levels of serum ALT, AST, total cholesterol, triacylglycerol, and low-density lipoprotein cholesterol. Histological analysis demonstrated that the extract ameliorated lipid accumulation. This effect was associated with the downregulation of the expression of cholesterol homeostasis-related genes (*ACC*, *FAS*, *SCD-1*, and *SREBP-1c*). Also, the extract inhibited hepatic ROS production and mitochondrial oxidative stress [57]. Therefore, the antihepatosteatosis activity of *M. nigra* might depend on its capability to inhibit the synthesis of fatty acids and cholesterol as well as its high antioxidant property.

The hepatoprotective effect of *M. nigra* has also been investigated in animal models of diabetes. In streptozotocin- and alloxan-induced diabetic rats, oral administration of the leaf extract can restore the serum levels of AST, ALT, triglycerides, and very low-density lipoprotein [58, 59]. The extract increases the glutathione level, decreases lipid peroxidation [59], restores the SOD/catalase balance [60], and improves histopathology of the liver [61].

The beneficial effects of *M. nigra* on the liver have also been shown in other study designs. In cell culture studies, the fruit juice, hydroalcoholic extract, and polyphenol extract of *M. nigra* showed a significant inhibitory effect on tert-butyl hydroperoxide-induced lipid peroxidation in isolated rat hepatocytes [12]. In D-galactose-induced aging mice, treatment with the leaf extract significantly increased SOD, catalase, and glutathione peroxidase activities and depressed lipid peroxidation in the serum, brain, and liver [62]. In a mouse with lipopolysaccharide-induced sepsis, treatment with the extracts of *M. nigra* leaves and pulp reduced the levels of TNF and increased glutathione content of the liver [63]. In rat model of diethylnitrosamine-induced hepatocellular carcinoma, *M. nigra* fruit extract exhibited a hepatoprotective effect through inhibiting oxidative stress and Wnt4/ β -catenin pathway and prolonged the survival of animals [64].

Potential Effects of *M. nigra* Phytochemicals on the Liver

It is well known that plant-derived phytochemicals can have beneficial effects on liver function. Some of these phytochemicals include flavonols, flavonolignans (e.g., silymarin), anthocyanins, and terpenoids [65–68]. As mentioned above, *M. nigra* is an applicable source of anthocyanins and flavonols. Potential mechanisms of hepatoprotective effects of *M. nigra* and its phytochemicals are shown in Figure 2. Hepatoprotective activities of flavonols (e.g., quercetin and kaempferol) have been reviewed elsewhere [66], and here we focus on anthocyanins. According to our thorough literature search, reported in Table 2, among *M. nigra* anthocyanins, cyanidin-3-glucoside, delphinidin, and pelargonidin play a major role in the hepatoprotective properties of this plant. In a rat model of diet-induced metabolic syndrome, cyanidin-3-glucoside reversed the increased plasma liver enzymes and enhanced inflammatory cell infiltration in the liver [69]. Protective effects of cyanidin-3-glucoside on alcohol-induced liver fibrosis were found to be mediated through regulating energy homeostasis and the AMP-ac-

tivated protein kinase/autophagy signaling pathway [70]. Also, cyanidin-3-glucoside could ameliorate CCl₄-induced liver injury (decrease of ALT and AST) in mice via increasing the activity of the antioxidant enzymes catalase, SOD, glutathione reductase, and glutathione peroxidase [71]. Also, this anthocyanin reduced hypercholesterolemia in mice by upregulating hepatic cholesterol 7 α -hydroxylase expression and activating the potential LXR α -CYP7A1-bile acid excretion pathway [72]. In the human hepatocyte cell line, it could enhance mitochondrial membrane potential and ATP production and up-regulate the gene expression of peroxisome proliferator-activated receptor-gamma coactivator 1-alpha, a transcription factor regulating mitochondrial function and biogenesis. Also, the expression of downstream PGC-1 α genes, mitochondrial transcription factor A, and nuclear respiratory factor 1 was increased as a result of treatment with cyanidin-3-glucoside [73].

Delphinidin as another major effective anthocyanidin of *M. nigra* is found to attenuate oxidative stress and restore hepatic architectures (by increasing matrix metallothionein I/II and metalloproteinase-9 expression) in CCl₄-induced liver fibrosis in mice [74]. Also, this compound reduces triglyceride accumulation in hepatocytes by modulation of lipid metabolic gene expressions [75].

Results of studies in mice with lipopolysaccharide-induced liver damage have shown that pelargonidin attenuates liver failure by inhibiting the toll-like receptor-mediated inflammatory pathway [76]. This anthocyanin ameliorated citrinin-induced oxidative stress in hepatocytes by modulating gene expression of the Keap1/Nrf2 pathway, the major signaling for regulation of cytoprotective responses to electrophilic and oxidative stress [77]. Pelargonidin also activated the aryl hydrocarbon receptor-CYP1A1 signaling pathway in hepatocytes [78]. This suggests it may interact with metabolisms of drugs in humans, which should be taken into consideration in future studies.

Toxicology of *M. nigra*

M. nigra has been used for thousands of years as a fruit and as a medicinal plant in traditional medicine. In the literature, toxicological data about chronic use of this plant are limited. Yilmaz and colleagues [79] used cultured human peripheral lymphocytes to study the potential genotoxic effect of *M. nigra* fruit. Their results demonstrated that the fruit juice had no genotoxic effect but

showed protective activity against mitomycin-induced genomic changes.

In the case of *M. nigra* leaves, safety reports are controversial. One study showed that oral administration of aqueous extract of mulberry leaves (100 mg/kg twice daily, for 42 days) caused a significant rise in serum ALT and AST activities in both normal and diabetic animals [80]. On the other hand, it has been reported that ethanolic extract of the leaves (up to 1,000 mg/kg, oral, for 28 days) did not induce toxic effects against renal and hepatic tissues [81]. Also, evaluation of the acute toxicity of ethanolic extract of the leaves showed no mortality and no toxicity signs at doses of 2 g/kg intraperitoneally and 5 g/kg orally in mice [82]. Therefore, according to the evidence reviewed above, it seems that acute and subacute consumption of *M. nigra* is not associated with significant toxic effects. However, the safety of chronic use of *M. nigra* should be investigated by further studies.

Conclusion

The present review summarized the hepatoprotective effects of *M. nigra* and its phytochemicals. Different parts of this plant particularly the fruit and leaf showed beneficial effects on hepatocytes in cell culture and animal models of liver damages [77]. Oxidative stress and inflammation are in the core of mechanisms of liver damages induced by viruses, drugs, or chemical agents. Although the exact mechanisms of hepatoprotective effects of *M. nigra* are a subject of further studies, the antioxidant and anti-inflammatory activities of this plant have been well documented. These activities can be attributed to the presence of considerable amounts of phenolic compounds such as anthocyanins, flavonols, and phenolic acids. The evidence reviewed in this study can help design clinical trials on *M. nigra* in patients with liver disorders and develop a hepatoprotective herbal medicine.

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Conflict of Interest Statement

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Author Contributions

Conceptualization and comprehensive review: A.G.; literature search, preparing tables, and writing original draft: S.H.; both authors have read and approved the submitted version.

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