Review Article
Curcumin and Liver Disease

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Abstract
Liver diseases pose a major medical problem worldwide and a wide variety of herbs have been studied for the management of liver-related diseases. In this respect, curcumin has long been used in traditional medicine, and in recent years it has been the object of increasing research interest. In combating liver diseases, it seems clear that curcumin exerts a hypolipidic effect, which prevents the fatty acid accumulation in the hepatocytes that may result from metabolic imbalances, and which may cause nonalcoholic steatohepatitis. Another crucial protective activity of curcumin, not only in the context of chronic liver diseases but also regarding carcinogenesis and other age-related processes, is its potent antioxidant activity, which affects multiple processes and signaling pathways. The effects of curcumin on NF-κB are crucial to our understanding of the potent hepatoprotective role of this herb-derived micronutrient. Because curcumin is a micronutrient that is closely related to cellular redox balance, its properties and activity give rise to a series of molecular reactions that in every case and biological situation affect the mitochondria. © 2013 BioFactors, 39(1):88–100, 2013

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1. Introduction
Liver diseases represent a major medical problem all over the world. In Europe and North America, alcohol abuse and overnutrition are the major causes of pathological conditions of the liver (with viral hepatitis increasing), whereas in Africa and Asia, the main concerns are viral and parasitic infections. Moreover, unlike other major causes of mortality, liver disease rates are increasing rather than declining [1]. The liver is the largest internal organ and it performs a great number of functions, supported by its different cell types [2,3]. Hepatocytes, present at the hepatic parenchyma, represent around 70% of the liver cell population. Hepatocytes are implicated in the secretion of proteins and bile, in cholesterol metabolism, and in the metabolism of glucose and glycogen. Detoxification, the metabolism of urea, acute phase response, and blood clotting are other functions performed by hepatocytes. Cholangiocyte or bile duct cells are present in the duct epithelium and represent around 3% of the liver cell population. They transport bile, control the rate of bile flow, and secrete water and bicarbonate to control the pH of bile. Endothelial cells are present at the liver vasculature, controlling blood flow and contributing to parenchymal zonation. Liver sinusoidal endothelial cells constitute around 2.5% of the lobular parenchyma and form the sinusoidal plexus to facilitate
blood circulation. They also enable the transfer of molecules and proteins between serum and hepatocytes, and are active in the scavenging of macromolecular waste, in cytokine secretion, antigen presentation, and blood clotting. Hepatic stellate cells (HSCs) are situated in a perisinusoidal position and represent around 1.4% of liver cells. Their functions are the maintenance of the extracellular matrix, vitamin A storage, control of vascular tone, contribution of regenerative response to injury, the secretion of cytokines, and to act as precursors of myofibroblasts. The activation of HSCs is the main event for cell-mediated mechanisms of liver injury [4]. Kupffer cells, which are present in the sinusoids and comprise around 2% of the liver, work by scavenging foreign material and secreting cytokines and proteases. Finally, pit cells are natural killer cells present in the liver; these are very rare and perform cytotoxic activity.

To understand liver pathology, it is necessary to briefly consider the structure of the adult liver. This organ might appear to be a mere pool of hepatocytes arranged within a low level histological structure. However, the liver has a very complex architecture organized around its basic unit, the liver lobule (Fig. 1). The liver lobule [5] is a hexagonal-like structure surrounding the central vein. Hepatocytes are arranged in arrays one or two cells thick. Sinusoids are special blood vessels formed between two arrays lined with sinusoidal cells connecting the portal venous system to the systemic venous system. Kupffer cells (liver-resident macrophages) are located in the sinusoids, and portal triads are arranged around the lobule. Triads consist of the termination of the portal vein branches, hepatic artery branches, and a bile duct. Between two hepatocytes, small bile ducts constitute the first passage for bile excretion by hepatocytes. Stellate cells are located in the space of Disse, between the hepatocytes and the sinusoidal cells.

A large number of molecules have been studied in attempts to manage liver-related diseases. Many of these drugs are of herbal origin and in some aspects they are still not well characterized. Curcumin has long been used in traditional medicine and research into this molecule has increased considerably in recent years. The main aim of this review is to summarize the most important actions of curcumin in liver diseases, focusing on its mechanism of action with respect to various pathological conditions. As another chapter of this book is devoted to curcumin and cancer, we will discuss here mainly non-neoplastic liver diseases.

### 2. Liver Diseases

The basic etiologies of liver injuries are presented in Table 1.

#### 2.1. Neoplastic Malignancies

Liver tumors, although not the most frequent, arouse great interest. Research in this field expands continuously, and significant advances are being achieved in diagnosis and treatment [6]. Hepatocellular carcinoma is one of the most common such malignancies and one of the principal complications in patients with chronic liver disease or cirrhosis related to hepatitis B or...
C virus infection [7,8]. Hepatoblastoma is a rare childhood cancer that presents low rates of incidence and is difficult to study. Nevertheless, different types and subtypes of hepatoblastoma have been identified [9,10]. Cholangiocarcinoma affects the biliary tract epithelium, and it may be present in intrahepatic or extrahepatic biliary ducts. Its incidence has increased in recent years, as have diagnostic mechanisms [11]. Primary hepatic angiosarcoma is a tumor of vascular endothelial cell origin. This represents only 1.8% of all hepatic diseases, and so it has not been as studied as much as other hepatic pathologies [12].

2.2. Alcohol-Related Disease
Alcoholism continues to affect ever more people worldwide, and it is a major cause of mortality in the United States and Europe. In disease prediction, practically all cases involve fatty liver (known as steatosis), because of the excessive accumulation of triglyceride in the hepatocytes. More than one in five alcoholics develop alcoholic hepatitis, and one-third of these will suffer cirrhosis, which may develop to hepatocellular carcinoma, depending on the intensity and duration of alcohol intake, gender, and genetic and epigenetic factors that affect alcohol metabolism and elimination in the liver. Alcoholic hepatitis takes place because alcohol compromises the intestinal barrier, leading to an increased presence of bacteria-derived lipopolysaccharide (LPS) in the portal blood, which produces an excessive and dysregulated inflammatory response, resulting in hepatocyte injury and tissue necrosis [13,14]. One of the main alcohol effects on hepatocytes is oxidative stress. Ethanol induces the generation of reactive oxygen substances (ROS) in hepatocytes, in the mitochondria, and also in cytosol, where free iron is present together with enzymes such as xanthine oxidase and aldehyde oxidase. Besides ROS production, alcohol products can damage hepatocyte antioxidant defense components, both enzymatic (such as superoxide dismutase, catalase, or glutathione transferase) and nonenzymatic (principally metal-binding proteins and vitamins) [13,14].

2.3. Hepatic Cholestasis
Hepatic cholestasis refers to the situation of damaged bile secretion in the liver, characterized by the blockage of bile flow from the hepatocyte to the intestine, with the consequent accumulation of hydrophobic bile acids in the liver and plasma. Its effects are commonly oxidative stress, increased apoptosis, and fibrosis [15]. Hepatic cholestasis is strongly related to mitochondrial dysfunction, because the mitochondria mediate death receptor signaling, contributing to oxidative damage and also to fibrosis, whereas inflammation has been linked with apoptosis [15]. Hepatic cholestasis is provoked by diverse inducing factors, including drug treatment, bile duct ligation, and inherited and syndromic forms [16,17]. Experiments with rats have shown that bile duct ligation perturbs liver homeostasis, raising levels of cytokines and signaling molecules; eventually, it may induce cirrhosis [18]. Inherited forms of intrahepatic cholestasis include disorders of primary bile acid synthesis and abnormalities in hepatocyte transport of bile constituents, due to gene alterations in synthesis or transporter mechanisms, such as progressive familial intrahepatic cholestasis syndrome, ATP8B1 disease, or ABCB4 disease. Other syndromic forms of inherited cholestasis include intrahepatic biliary hypoplasia, familial hypercholanemia, or lymphedema-cholestasis syndrome [13].

2.4. Nonalcoholic Fatty Liver Diseases
Nonalcoholic fatty liver diseases (NAFLD) range from hepatic steatosis, the most clinically benign, through nonalcoholic steatohepatitis (NASH), an intermediate lesion, to cirrhosis. Steatosis, NASH, fibrosis, and cirrhosis can result from metabolic abnormalities and/or genetic predisposition, whereas NAFLD is associated with obesity, insulin resistance, and type 2 diabetes. Studies have suggested that obesity-related fatty liver disease is related to metabolic syndrome [19,20]. NASH, the most widely studied NAFLD and which was first described as a clinical entity by Ludwig et al. [21], is a precursor to more severe liver disease, and in almost 25% of patients it evolves into cirrhosis or even hepatocellular carcinoma. In addition, it represents a frequent cause of abnormal liver test results in blood donors, and is responsible for the asymptomatic elevation of serum aminotransferases in over half of cases [22].

The histological features of NASH have been well described, and in 1999, Brunt et al. [23] proposed a system to classify the global assessment of necroinflammatory activity (grade) and fibrosis with or without architectural remodeling (stage). This classification included three categories or grades of NASH necroinflammation: grade 1 (mild), grade 2 (moderate), and grade 3 (severe), which correlate, respectively, with hepatocellular steatosis, ballooning, and disarray, together with inflammation (acinar and portal). According to this classification, fibrosis is constituted of four categories (stages 1–4), depending on the increase in connective tissue deposition and architectural remodeling. However, the pathogenesis of NASH is still not fully understood. The theory currently most accepted is “the two-hit hypothesis,” proposed by Day and James [24]. The “first hit” is the development of steatosis, following prolonged overnutrition that deregulates the lipid metabolism and provokes an accumulation of free fatty acids (FFAs) and triglycerides in the liver. The increased presence of FFAs in hepatocytes reduces β-oxidation, and thus enhances the accumulation of fatty acids. In the “second hit,” steatosis progresses to inflammation and fibrosis due to oxidative stress, mitochondrial dysfunction, and inflammatory cytokines, leading to liver cell inflammation and necrosis, and activating the fibrogenic cascades [20,22,25].

2.5. Human Hepatitis Viruses
There are five human hepatitis viruses, A–E, each with a different development of infection. Hepatitis A and E are always transient, whereas Hepatitis B, C, and delta may be either transient or chronic. Despite their differences, they all have important features in common: their primary target of infection is the hepatocyte, where they infect and replicate,
although the mechanisms of viral infection and pathogenesis vary depending on the hepatitis virus [13].

During the acute phase of hepatitis, for 2–6 weeks, many hepatocytes are infected and viruses may enter the bloodstream or the bile canaliculi. In this acute phase, the immune system attempts to eliminate the virus, primarily by antibodies specifically directed against viral antigens. In a second step, intracellular viruses are eliminated by the immune response, via the immune destruction of infected cells by cytotoxic T lymphocytes (CTLs). This second step can also be achieved by noncytolytic elimination, by antiviral cytokines that inhibit viral gene expression and replication, and which therefore do not need to destroy the infected cell. Chronic liver disease appears when the immune system fails to eliminate the virus infection [13]. Hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis delta virus (HDV) are potentially the most harmful for the liver, being responsible for a large proportion of liver failure conditions, such as fulminant hepatitis, cirrhosis, and hepatocellular carcinoma [26]. HBV infection can be transient, in the form of acute hepatitis or fulminant hepatitis, or develop to become chronic, leading to cirrhosis and/or hepatocellular carcinoma [27]. Regarding HCV, up to 75% of persons infected progress to chronic hepatitis C. Most of these patients are asymptomatic and present no physical signs of chronic liver disease, other than fatigue. Chronic hepatitis C often progresses to cirrhosis and hepatocellular carcinoma [7,13].

2.6. Hepatic Toxicity

More than 1,000 drugs can induce hepatic toxicity, and the risk increases when the daily dose is higher than 50 mg or when the liver is highly involved in metabolizing the drug. The principal mechanism of drug-induced liver damage is that of mitochondrial dysfunction and lipid dysmetabolism, caused by the drug itself and/or by the reactive metabolites generated. Mitochondrial dysfunction can have other harmful consequences, such as oxidative stress, energy deficiency, steatosis by accumulation of triglycerides, and cell death. In association with obesity and diabetes, drugs may induce acute liver injury, steatosis, and even steatohepatitis, which can lead to cirrhosis [28].

Another type of hepatic toxicity is iron induced. Iron is an essential constituent of the body that participates in a large number of biological reactions. It is present in functional form in molecules such as cytochromes, hemoglobin, myoglobin, and iron-dependent enzymes, and a good iron balance is necessary to avoid diseases. Iron overload may be caused by a wide range of acquired and hereditary conditions, such as hereditary hemochromatosis [29], and this condition is associated with various toxic effects, of which the most common is liver damage, with the excessive deposition of iron giving rise to fibrosis and cirrhosis [18].

The capacity of the liver to metabolize and help excrete xenobiotics makes it very susceptible to damage, which may be reversible but may also lead to fulminant hepatic failure and death. The administration of high doses of thioacetamide (TAA) to rats causes fibrosis, whereas cirrhosis is provoked by periodic lower doses, accompanied by high levels of ROS, the production of proinflammatory molecules, and the activation of HSCs [18,30].

In humans and animals, hepatotoxicity is the major outcome of exposure to carbon tetrachloride (CCl4). Liver injury is detectable by clinical signs (jaundice, swollen, and tender liver), biochemical alterations (elevated levels of hepatic enzymes in the blood and loss of enzymatic activities in the liver), or histological examination (fatty degeneration and necrosis of central hepatocytes, destruction of intracellular organelles, fibrosis, and cirrhosis). In the absence of clinical signs, elevated levels of serum enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and gamma glutamyl transferase may provide evidence of hepatocellular injury [31].

2.7. Hepatic Fibrosis, Cirrhosis, and Portal Hypertension

Many of the above pathological conditions can lead to hepatic fibrosis and cirrhosis or are closely related to portal hypertension-related events. Fibrosis is characterized by an excessive accumulation of extracellular matrix proteins, including collagen. The main collagen-producing cells in the liver are HSCs, which can be stimulated by ROS, inflammatory cytokines, and apoptotic signals, among others. When this occurs, the hepatic architecture is altered; a fibrous scar appears and nodules of regenerating hepatocytes develop. If the damage continues, the hepatic fibrosis advances to become hepatic cirrhosis, which cannot be reversed. However, recent experimental findings suggest that even advanced fibrosis may be reversible [13,17,32,33]. Cirrhosis is the last pathological feature of chronic hepatic injury and is characterized by the destruction of the hepatic architecture and vascular structures, together with blood flow failure due to the excessive deposition of collagen in the cirrhotic liver, resulting from an imbalance between collagen synthesis and degradation by membrane-bound metalloproteinase [17,32,33]. Portal hypertension in the liver is the hemodynamic disorder that is most frequently associated with cirrhosis. Cirrhosis causes distortion of the hepatic architecture, followed by increased intrahepatic resistance and, finally, portal hypertension, which is associated with other clinical pathologies that may affect diverse organs. Such pathologies include digestive hemorrhage, hypertensive gastropathy, hepatorenal and hepatopulmonary syndromes, and alterations in the elimination of drugs, microorganisms, and chemical substances by the liver [13,34]. In addition to changes in liver architecture caused by cirrhosis, the first step to portal hypertension is an increased resistance to portal blood flow due to deficit and hyporesponse to vasodilators, such as nitric oxide (NO), carbon monoxide (CO), or hydrogen sulfide (H2S). The increased production of and response to vasoconstrictors, such as endothelin, angiotensin II, or alpha-adrenergic stimulus, also promotes hepatic vascular resistance. This imbalance between excessive vasoconstrictors and deficient vasodilators leads to an increase in portal blood flow that aggravates portal hypertension [13].
3. Mechanisms Involved in Liver Disease

Various in vitro and in vivo observations suggest that oxidative stress and associated DNA, protein, and lipid damage may underlie liver diseases as a key pathophysiological force and which may also be related to chronic liver injury, hepatic inflammation, and fibrosis [35].

Oxidative stress is a physiological consequence of the disturbances in the oxidative balance caused by the interplay between the production of free radicals and the activity of the antioxidant defense system. Free radicals are inherent to aerobic life, and are produced and involved in important cellular processes such as the immune response, vascular biology, thyroid hormone biosynthesis, and oxygen tension sensing. Consequently, they are key mediators of physiological cell activity; however, when the production of free radicals exceeds the capacity of the antioxidant system to cope with oxidative cellular and tissue damage, oxidative stress occurs. Diverse consequences arise from persistent oxidative stress, because free radicals alter the chemical structure of any cell component but they also play a crucial role in cell signaling as second messengers. Thus, an imbalance in the cellular and/or extracellular concentration of free radicals contributes significantly to organ injury and dysfunction, and may ultimately lead to life-threatening degenerative diseases [36].

Among the latter, non-neoplastic liver diseases secondary to inflammation and fibrosis are evidently connected with chronic oxidative stress. Liver fibrosis and its end-stage cirrhosis are the consequences of chronic liver injury of varied etiology, including the liver diseases summarized in Section 2. Proinflammatory insults originate from viral, toxic, metabolic, or autoimmune processes and lead to the activation of HSCs, which transdifferentiate from a quiescent lipid-storing phenotype to an α-smooth muscle actin (α-SMA) positive phenotype. Activated HSCs are myofibroblast-like cells (MFs) that favor extracellular matrix deposition materials, mainly collagen I, as part of the liver’s wound healing mechanism. Portal fibroblasts and bone marrow-derived mesenchymal stem cells are also transdifferentiated into MFs by chronic liver injury. Together, these cells originate a homogeneous proliferating, contractile, and fibrogenic MF cell population regardless of its cellular origin, in terms of activation and functionality [37]. MFs also contribute to liver fibrosis by releasing profibrogenic and proinflammatory cytokines and tissue inhibitors of metalloproteases [38,39]. This progressive deposition of extracellular matrix leads to hepatic structural and functional impairment.

3.1. Oxidative Stress and Inflammation

Among the mechanisms involved in mediating the process of liver fibrosis, an important role is played by those mediated by ROS, because persisting liver injury leading to parenchymal damage and hepatocyte loss induces the recruitment of immune effectors at the injured site. Activated resident Kupffer cells and blood-derived neutrophils, monocytes/macrophages, and lymphocytes all release growth factors, cytokines, chemokines, and ROS, which in turn activate HSCs into becoming MFs [37,40].

Early MF activation involves the rapid induction of β-platelet-derived growth factor (β-PDGF) receptor, the development of a contractile and fibrogenic phenotype, and the modulation of growth factor signaling. The autocrine PDGF loop is among the most potent of all cytokine loops during HSC activation. The union of PDGF with its receptor activates focal adhesion kinase (FAK), phosphatidylinositol 3-kinase (PI3K), and extracellular regulated kinase (ERK) signaling, and hence HSC proliferation [41]. PDGF signaling is sustained through a positive feedback mechanism that involves ROS. When the PDGF binds to its receptor, PI3K and Rac are activated, and this in turn activates nonphagocytic membrane NADPH-oxidase (NOX) and ROS production. Among other mitogens that promote HSC proliferation and activation through NOX activation, those of vascular endothelial growth factor (VEGF), thrombin and its receptor, interleukin (IL)-1, epidermal growth factor (EGF), angiotensin II (Ang II), and basic fibroblast growth factor (bFGF) are very significant [42–44]. Other important mitogens for HSCs are tumor necrosis factor λ (TNFλ) and IL-6, which induce differentiation and activation of the HSCs through the activation of a redox-sensitive transcription factor nuclear factor κB (NF-κB), which also plays a prominent role in liver inflammation, as discussed below [44].

A second event in HSC activation involves migration toward injury sites, driven by chemotacticants including PDGF and VEGF, which induce the formation of cell protrusions through protein kinase D1 [45], cytoskeletal reorganization through protein kinase C (PKC) and Rho-dependent signaling [46], CXCR3 ligands by activation of the Ras/ERK cascade [47], and monocyte chemotactant protein 1 (MCP-1) to recruit and activate monocytes [48]. MCP-1 is mainly involved in the formation and maintenance of the inflammatory infiltrate in various wound-healing conditions, including chronic liver diseases. MCP-1 is overexpressed by a wide range of hepatic cells including injured hepatocytes [49], activated macrophages and Kupffer cells, activated MFs, and activating HSCs [50] subsequent to the induction of redox transcription factors such as NF-κB and activation protein 1 (AP-1) [51]. Although HSCs and activated MFs play a significant role in chronic liver diseases because of their highly fibrogenic activity, a wide variety of immune and inflammatory cells are also recruited to injury sites, forming the inflammatory infiltrate, including mononuclear cells, Kupffer cells, sinusoidal endothelial cells, platelets, T-lymphocytes, and endothelial progenitor cells [52]. As mentioned above, mediators of oxidative stress, such as ROS and other reactive species, may contribute to injury continuity, by triggering and regulating the expression of proinflammatory cytokines and chemokines in MFs and immune cells. The main signaling pathway inducing this activity is mediated by the activation of NF-κB, a major redox-sensitive transcription factor, which binds to specific DNA regions known as κB.
sequences and activates the expression of genes involved in inflammation, cell survival, proliferation, and differentiation in response to different stimuli, including the presence of free radicals [53]. The injury-induced activation of hepatic NF-κβ promotes the production and secretion of TNFα, IL-6 in Kupffer cells [52] and the expression of inducible NO synthase (iNOS) and cyclooxygenase-2 in monocytes [54]. Generally, any cytokine capable of activating NF-κβ is likely to induce ROS generation, which further increases the inflammatory response. On the other hand, oxidative products such as 4-hydroxy-2,3-nonenal have been shown to upregulate the expression of tumor growth factor (TGF-β1) in Kupffer and macrophages [55] and to promote leukocyte chemotaxis [56]. Additionally, intrahepatic hypoxia may occur during inflammatory and fibrotic processes, and indeed, the role of proangiogenic cytokines and the expression of related receptors in liver fibrosis is a determinant factor in advanced liver cirrhosis [57]. At this stage, the role of injured hepatocytes, which are prone to apoptosis and necrosis through mitochondrial impairment and ROS release, is of crucial importance for injury perpetuation and liver disease progression to cirrhosis.

3.2. Apoptosis

As mentioned above, with parenchymal damage and inflammatory signaling, the injury focus is a complex and highly oxidative environment composed of inflammatory and angiogenic cells releasing a wide variety of growth factors and inflammatory cytokines and chemokines. Among the latter, TNFα activates molecular pathways, which leads to either pro-survival or to proapoptotic and necrotic signals, depending on the redox state of the hepatocyte.

The interaction of TNFα with its type 1 receptor (TNFR1) is followed by the association of the TNF-receptor-associated death domain (TRADD), the Rieske iron-sulfur protein of mitochondrial ubiquinol-cytochrome c reductase (RIP1), and TNF-receptor-associated factors 2 and 5 (TRAF-2 and TRAF-5). These interactions activate NF-κβ and AP-1 transcription factors, which in turn activate the expression of genes that promote hepatocyte survival [58]. However, glutathione (GSH) depletion alters the susceptibility of hepatocytes to TNF-α and promotes the TNF-α-induced apoptosis axis, which finally leads to mitochondrial dysfunction, promoting the overproduction of ROS, impaired bioenergetics, severe oxidative stress, and cell death [59,60]. Under these circumstances, the TRADD/RIP-1/TRAF-2 complex can dissociate from TNRF1 and bind Fas ligand-associated death domain (FADD), recruiting caspase 8/10, which cleaves the proapoptotic protein BH3 interacting domain death agonist (BID). The cleaved BID form translocates to the mitochondria, producing the permeabilization of the mitochondrial outer membrane, the release of cytochrome c, and the activation of the classic or intrinsic apoptosis pathway and ROS production [41,61].

Alternatively, upon TNFα stimulation, RIP1 may be capable of translocating to the mitochondria and permeabilizing the mitochondrial outer membrane, thus eliciting ROS release, without cytochrome c release, provoking necrotic and caspase-independent cell death [62]. Upon these events, the ROS-mediated and sustained activation of c-Jun amino-terminal kinases (JNKs), through the oxidative inhibition of JNK phosphatases, may also reinforce hepatocyte apoptosis [59].

Severe oxidative stress secondary to an acute inflammatory response in liver injury may then provoke a molecular shift that involves the transition from survival signals to death signals in injured hepatocytes. This in turn releases further ROS to the injury site, reinforcing the inflammatory response and activating HSCs in a paracrine fashion. The result is a chronic inflammatory process that contributes to perpetuate fibrogenic progression through sustained MF activation.

3.3. Fibrogenesis

Fibrogenesis is the event preceding severe liver malfunction. Activated MF cells produce and deposit large amounts of collagen type I fibers and, to a much lesser but still significant extent, collagen type III at the liver injury site. Fibrogenic activity is mainly mediated by TGFβ-1, which typically acts by binding TGFβ type II receptors. Subsequent TGFβ type I activation and Smad protein association and phosphorylation result in a heterodimeric complex that translocates to the nucleus and binds to specific nucleotide motifs at the promoter region of the collagen, type I, alpha 1 (COL1A1) gene [63]. Additionally, TGFβ-1 induces the accumulation of hydrogen peroxide (H2O2), which is involved in upregulating the expression of the COL1A1 gene through a CCAAT/enhancer binding protein-β (C/EBPβ)-dependent mechanism in MFs [64]. The TGFβ-1 fibrogenic signal is also mediated by connective tissue growth factor (CTGF), which is normally expressed by MFs, endothelial cells, and ductular epithelial cells but also by parenchymal hepatocytes [65]. Moreover, CTGF expression in parenchymal hepatocytes appears to be both TGFβ-1-dependent and TGFβ-1-independent, and this process can also be upregulated by a wide variety of factors, including lipid peroxidation products (hydroxynonenal and malondialdehyde) [66].

Finally, the contraction of active fibrogenic MFs may contribute to intrahepatic resistance, portal hypertension, and lobular distortion as fibrosis advances and cirrhosis develops. MF contraction activity is mediated by both Ca2+-dependent kinases such as the myosin light chain kinase (MLCK) pathway, and by Ca2+-sensitization mechanisms such as the myosin light chain kinase (MLCK) pathway, and by Ca2+-dependence mechanisms such as 17-kDa PKC-potentiated protein phosphatase 1 inhibitor protein (CPI-17) and myosin light chain phosphatase targeting subunit 1 (MYPPT1) pathways [67].

From this summary of the most active and important signaling pathways that intervene in liver fibrosis, it is possible to envisage a highly oxidative environment in which an inducible and complex cell population receives a wide range of signals from the extracellular milieu, such as growth factors, cytokines, and ROS or other reactive species, which promote inflammatory and fibrogenic processes. In such a highly inducible and oxidative microenvironment, immune and angiogenic cells unavoidably increase their intracellular concentration of...
ROS, which significantly affects cellular signaling pathways, increasing disease severity and finally leading to liver degeneration and cirrhosis. In this sense, it is well known that protein tyrosine phosphatases (PTPs) are susceptible to oxidation because of chemical changes in the conserved cysteine residue of their catalytic domain that possesses a low pKₐ. This reaction inhibits phosphatase activity, and thus increases cytoplasmic protein kinase activity and stimulates the wide range of intracellular signaling cascades that are mediated. Among these molecular pathways, those initiated by inflammatory cytokines and growth factors can be counted [68,69]. These important changes affect MP and inflammatory cells, as well as hepatocytes, increasing profibrotic signaling and reinforcing ROS-mediated signaling in a cyclic fashion. With respect to the latter, mitochondrial damage plays a pivotal role, acting as an “amplifying effector” in a key stage of liver disease, and making a major contribution to the transition from acute injury to chronic damage through hepatocyte cell death and ROS release.

3.4. Hyperlipemia
As stated above, a high level of circulating and hepatic FFAs is a common cause of liver steatosis and steatohepatitis. Metabolic disturbances leading to steatosis (as associated with obese or overweight patients and with metabolic syndrome, and often including diabetes, insulin, and leptin resistance) lead to a long-term accumulation of triglyceride in the hepatocytes, coupled to ROS production, due to mitochondrial impairment. This process is described in the “two hit” model of NASH published by Day and James, which continues to be widely accepted [24]. Mitochondrial dysfunction leads to altered bioenergetics through the impairment of mitochondrial β-oxidation and FFA accumulation, which in turn induce microsomal FFA oxidation (ω-oxidation) involving cytochrome P450 isoforms CYP2E1 and CYP4A to compensate for FFA overload. CYP2E1 and CYP4A activity induces the production of ROS, increasing intracellular oxidative stress and leading to lipid peroxidation [70]. It has also been shown that ROS generated by CYP2E1 activity promotes insulin resistance, decreases insulin signaling in the liver, and increases hepatic fat accumulation [71].

The mitochondrial β-oxidation of FFA continues in parallel and it is not inhibited until massive electron flux on the electron transport chain and ROS overproduction provoke mitochondrial dysfunction. It has been shown that mitochondrial lipotoxicity is not an early event due to hepatic hyperlipidemia; indeed, initial transient increased mitochondrial activity has been reported in mice fed with hyperlipidic diets; this is then followed by a significant decrease in oxidative phosphorylation due to mitochondrial impairment [72,73]. As mitochondrial oxidant capacity falls and ROS production increases, lipids accumulate and lipid peroxidation takes place, further increasing oxidative stress and cellular damage. Under these highly stressing circumstances, mitochondrial uncoupling seems to take place. Uncoupling protein-2 (UCP-2) is thought to activate increasing proton leaks across the mitochondrial inner membrane, reducing mitochondrial inner transmembrane potential (∆Ψᵢₘ) and oxidative phosphorylation. This protective effect against ROS production may in turn favor further triglyceride accumulation in the hepatocytes. This topic remains controversial in mitochondrial bioenergetics, and further data are required to clarify the question [74–76].

Many of these changes are mediated by the activation of nuclear peroxisome proliferator-activated receptor-γ (PPAR-γ), which promotes the expression of enzymes involved in lipid catabolism in the mitochondria and in the peroxisomes. Interestingly, PPAR-γ is activated by long-chain FFA, which may be the cause of the observed rise in the expression of this transcription factor in obesity and steatosis models [77,78].

Thus, defects in lipid homeostasis, bioenergetic impairment, and the overproduction of ROS, leading to lipid accumulation and oxidative stress due to severe hyperlipemia, all induce massive hepatocyte apoptosis, which provokes an acute inflammatory response, progressing to liver necrosis, increased and chronic inflammation, and liver fibrosis. NASH and NAFLD are classic examples of chronic liver diseases induced by hyperlipemia.

4. Curcumin in Liver Diseases
Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is a natural yellow polyphenol extracted from the rhizome of turmeric (Curcuma longa), a plant grown in tropical and subtropical regions throughout the world, which is extensively used for food preparation in many Asian countries. Besides its culinary use, curcumin has been considered a medicinal herb in the traditional medicine of several countries for centuries, because of its antioxidant, anti-inflammatory, antimitagenic, antimicrobial, and anticancer properties. However, the molecular network that governs curcumin-derived effects on health has not been completely elucidated [79].

Numerous in vitro studies have indicated that curcumin exerts potent antioxidant and anti-inflammatory properties, which may account for its protective effect in chronic liver diseases. Importantly, several in vivo studies from animal models of chronic liver diseases have shown that curcumin possesses antifibrogenic properties, mediated by the inactivation of different processes involved in liver damage [80].

Early experiments published by Matsuda et al. [81,82] showed the hepatoprotective role of the main sesquiterpenes isolated from the aqueous acetone extract of Zedoaria Rhi- zoma, the rhizome of Curcuma zedoaria, in an acute liver injury mouse model induced by ν-galactosamine (ν-GalN)/LPS toxicity. In vitro and in vivo experiments suggested that curcu- min may play a major role in hepatocytes protection by inhibiting the production of NO and TNF-α in LPS-activated Kupffer cells. Later studies using LPS-induced hepatotoxicity further sustained these findings, highlighting the interfering role of
curcumin on the inflammatory and apoptotic TNF-α/NF-κB signaling pathway. Lukita-Atmadja et al. [83] showed that pretreatment with curcuminoids of endotoxemic BALB/C mice significantly reduced the phagocytic activity of Kupffer cells and suppressed the hepatic microvascular inflammatory response to LPS. On the basis of their histopathologic observations and previous experimental data, the authors hypothesized that curcumin anti-inflammatory effects may be mediated by the inhibition of the nuclear translocation of NF-κB and its dependent proinflammatory cytokines, what may contribute to the significant decrease in neutrophils recruitment to portal and central venules as well as in the sinusoids of mice treated with curcumin with respect to control mice. Later on, Kaur et al. [84] and Yun et al. [85] provided further insights into the molecular mechanisms of curcumin-derived protection against LPS- and LPS/GaIN-induced liver injury, respectively, confirming the involvement of TNF-α inhibition in these processes. Both works reported that curcumin administration before induction with GaIN/LPS or LPS alone prevented the elevation in serum TNF-α and aminotransferases, reduced hepatic necrosis, and inflammation. Additionally, Kaur et al. confirmed the antioxidant effect of curcumin by reporting decreased levels of lipid peroxidation measured as thiobarbituric acid reactive substances (TBARS) and increased GSH and SOD levels in the liver homogenates from LPS-challenged rats supplemented with curcumin. In 2011, Černý et al. [86] contributed with a work showing that the ameliorative effects of curcumin on liver injury in a rat LPS/dGalN model were also mediated by the positive regulation of antioxidant and cytoprotective enzymes, such as heme oxygenase-1 (HO-1). On the other hand, GalN/LPS treatment dramatically increased lipid peroxidation levels, NO production, and the expression of NO synthase 2 (NOS-2) in the serum and liver of experimental animals. Curcumin-derived hepatoprotection was evidenced through the decrease in serum aminotransferases and the elevation of the expression and activity of HO and the acute drop in mRNA levels of NOS-2, NO, both in plasma and liver, and liver CO, which is a product of lipid peroxidation. Increases in plasma bilirubin normally reflect the severity of hepatic injury upon hepatotoxic GalNLPS treatment, but, in this work, the authors argue that further increases in the experimental group supplemented with curcumin beyond those observed in rats not supplemented with curcumin are the consequence of HO-1 activity. Interestingly, bilirubin is a potent antioxidant preventing lipid peroxidation (which indeed demonstrated to be reduced upon curcumin treatment). On the other hand, previous in vitro studies using RAW264.7 macrophages have suggested that HO-1 upregulation and the decrease in NO production are mediated by the inhibitory effect of curcumin on NF-κB [87], and the use of monocarbolyl analogs of curcumin has also showed to decrease LPS-induced expression of TNF-α and IL-6 in mouse J774A.1 macrophages [88].

Apart from those works using LPS/dGalN-induced hepatitis models, many other in vitro and in vivo experiments, performed in a wide variety of liver injury models, have evidenced the protective activity of curcumin and confirmed the definitive involvement of its effects on NF-κB signaling, which remain paramount to our understanding of the potent hepatoprotective role of this herb-derived micronutrient.

Ramirez-Tortosa et al. [22] showed that curcumin supplementation to experimental rabbits with high-fat-induced NASH lowers the grade of NASH and aminotransferase activity, and raises levels of mitochondrial antioxidants with respect to healthy control animals. In addition to these changes, curcumin increased levels of mitochondrial antioxidants, reduced mitochondrial reactive oxygen species, improved mitochondrial function, and lowered levels of TNF-α protein levels, thus inhibiting the key apoptotic TNF-α/NF-κB axis in hepatocytes during initial phases of the inflammatory infiltrate formation followed by massive HSC activation and fibrosis. Curcumin treatment has also been shown to decrease hepatic NF-κB levels, markers of hepatic inflammation, and hepatomegaly in diabetic and obese mouse models [89]. A significant inhibition of NF-κB activity is also the main mechanism by which curcumin has been shown to preserve liver tissue integrity in early stages of hepatic fibrosis in alcoholic liver injury rats [90]. In a rat model of TAA-induced hepatotoxicity, curcumin has demonstrated to improve the survival rates of the animals by significantly inhibiting the nuclear binding of NF-κB and the iNOS protein expression [91]. Novel formulations of curcumin, as NanoCurc™, showing enhanced bioavailability in liver tissue, have rendered lower mRNA levels of TNF-α and IL-6 and enhanced antioxidant capacity in liver tissue from mice injected with CCl₄ intraperitoneally [92]. Curcumin supplementation of mice fed with the methionine- and choline-deficient diet, which develop steatohepatitis, also showed decreased NF-κB activation and induction of downstream inflammatory cytokines [93]. In vivo experiments with hamsters infected with the trematode Opisthorchis viverrini, a liver fluke, and treated with praziquantel have showed the induction of several inflammatory cytokines, namely NF-κB, and downstream effectors such as TNF-α, IL-1β, iNOS, and COX-2, and enhanced levels of oxidative stress, which are significantly ameliorated after treatment with curcumin [94]. Experimental liver steatosis induced by TNF-α injection in mice showed significant attenuation after curcumin treatment reflected in decreased oxidative stress, neutrophils infiltration, and improved histopathology [95]. Taking into account the heterogeneous sources of liver damage in the models employed by these studies and their invariable confluence on the molecular pathways affected by curcumin in the reduction of damage, it should be pertinent to conclude that the inhibition of the NF-κB axis plays a major role regarding the hepatoprotective activity of curcumin, yet its specific molecular mechanisms remain to be elucidated.

As mentioned before, besides TNF-α signaling, curcumin has been reported to inhibit the expression of other NF-κB-dependent inflammatory chemokines and cytokines, such as IL-6, IL-2, TGF-β, and MCP-1, and inflammation-promoting enzymes such as COX-2 and iNOS in Kupffer cells and hepatic tissue homogenates [92,94,96,97]. Particularly, TGF-β is of
special interest during the latest phase of liver injury given its involvement in liver fibrosis. In vivo studies using a bile duct ligation and CCl₄ rat models have shown that curcumin is able to partially inhibit the expression of TGF-β, significantly preventing bile duct ligation fibrosis [98]. Using the rat HSC-T6 cell line, Lin et al. [99] showed that curcumin suppressed the expression of SMA and collagen deposition in the HSC-T6 cells after stimulation with TGF-β, accounting for its antifibrogenic effects. Nevertheless, increased curcumin concentrations elicited a different effect, as they induced HSC-T6 cells apoptosis. Then, it has been suggested that curcumin exerts its hepatoprotective effects at this level through different and dose-dependent mechanisms. This observation was further corroborated in vivo, through a study published shortly after by Shu et al. [100] using a CCl₄-induced liver injury rat model. Later, Nakayama et al. [101] studied the molecular processes governing TGF-β-mediated liver fibrosis and curcumin injury prevention using human Hep2G cells. This approach showed that TGF-β induces the expression of plasminogen activator inhibitor type-1 (PAI-1), a highly profibrotic molecule, through multiple mechanisms involving NF-κB signaling and oxidative stress. Interestingly, the authors argued that curcumin significantly reduced PAI-1 expression, providing a mechanistic explanation for the hepatoprotective effects of curcumin regarding liver fibrosis. Additionally, curcumin has been related to a significant reduction in liver fibrosis and injury induced by both CCl₄ and Concanavalin A through the negative modulation of the expression of Toll-like receptor (TLR) 2, TLR4, and TLR9, providing further insights into the molecular processes involved in liver pathogenesis [102,103].

Curcumin’s activity, which effectively modulates important molecular pathways for cell biology and homeostasis, shows the therapeutic potential of this widely appreciated micronutrient, not only in the context of chronic liver diseases but also regarding carcinogenesis and other age-related processes. Beyond to its known attenuating role in inflammation and fibrosis signaling, curcumin is well known to exert a potent antioxidant activity. Indeed, many of the works discussed above include the analysis of several oxidative stress and antioxidant markers to further elucidate the molecular mechanisms by which curcumin elicits its hepatoprotective activity.

Curcumin has been shown to improve both acute and subacute liver injury induced by CCl₄ intraperitoneal injection in rats by lowering the activity of serum aminotransferases and the levels of liver lipid peroxidation [104], reactivating the impaired activity of important antioxidant enzymes, such as hepatic SOD, lowering liver lipids and lipid peroxidation, and increasing total GSH levels [105] Similarly, Pari et al. showed that curcumin and tetrahydrocurcumin, one of the main products of curcumin catalysis, administered before chemical induction of hepatocellular damage with erythromycin estolate or chloroquine effectively reduce serum and hepatic levels of lipid peroxides and TBARS together with a significant increase in nonenzymatic and enzymatic antioxidants [106,107]. Importantly, curcumin is known to inhibit GSH depletion, which is frequently observed in both experimental alcoholic liver disease and in CCl₄-induced injury [108,109]. Increased levels of catalase, glutathione peroxidase, and GSH are also restored after curcumin supplementation in hepatocellular toxicity models induced by cadmium [109,110] or paracetamol [111,112].

Curcumin has been shown to decrease lipid storage in the hepatocytes of high-fat diet rodents, contributing to minimize the liver damage induced by mitochondrial dysfunction and oxidative stress. Initial data regarding the hypolipemic activity of curcumin were reported by Rao et al. [113], who showed that rats fed concurrently with cholesterol and curcumin presented a lower cholesterol content in the liver than did rats fed with cholesterol only. Later, Asai and Miyazawa [114] showed that male Sprague-Dawley rats supplemented with 1.0 g curcuminoids/100 g diet registered significantly lower liver triacylglycerol and cholesterol concentrations and significantly higher hepatic acyl-CoA oxidase activity than did control rats not given any curcumin supplement. This would account for curcuminoids’ lipid-lowering potency in vivo. Rukumani et al. [115,116] assessed the hepatoprotective and hypolipemic effects of curcumin and its synthetic analogs in a male rat model of alcohol-induced and high-fat diet liver injury. In both experiments, curcumin was shown to attenuate histopathological changes in the liver and to reduce hepatic tissue levels of cholesterol, triglycerides, and FFAs. More recently, Jang et al. [117] showed that curcumin supplementation in a high-fat diet in hamsters lowered levels of circulating lipids and hepatic lipids. Significantly, they also showed that curcumin increased fatty acid β-oxidation activity and reduced the activity of lipid anabolic enzymes such as fatty acid synthase, 3-hydroxy-3-methylglutaryl CoA reductase, and acyl CoA cholesterol acyltransferase. The latter property was also highlighted very recently by Shao et al. as a molecular mechanism of curcumin hepatoprotection [118]. Consequently, decreased levels of hepatic lipid peroxides were detected in hamsters given a diet supplemented with curcumin, with respect to control hamsters. Finally, curcumin has also been shown to significantly inhibit the expression and activity of key proteins involved in metabolism, such as hepatic protein tyrosine phosphatase 1B (PTP1B), which is associated with defective insulin and leptin signaling in a model of hypertriglyceridemia and hepatic steatosis in fructose-fed rats [119], and leptin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) as a result of the disruption of Wnt signaling and the activation of PPAR-γ. Reducing the expression of LOX-1, curcumin has been shown to block the transport of extracellular ox-LDL into HSCs, thus contributing to preventing their activation [120]. It seems clear that curcumin exerts a hypolipidic effect, which prevents fatty acid accumulation in the hepatocytes as a result of various metabolic imbalances that finally lead to NASH.

Finally, very recent studies have reported that curcumin also inhibits CYP2E1 activity [121], thus limiting ROS production from microsomes, and activates NF-E2-related factor 2 (Nrf2) translocation to the nucleus, where it activates the.
expression of antioxidant enzymes [94,122], elevating the expression of the apurinic/apyrimidinic endonuclease1/redox factor-1 (APE1/Ref-1) DNA repair protein [97], and preventing the activation of HSCs through PPAR-γ overexpression, which maintains lipid storing (quiescent) phenotype in these cells and blocks PDGF and EGF signaling [123]. It also increases the expression of mitochondrial biogenesis genes such as nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (Tfam) [124].

Regarding clinical studies assessing the hepatoprotective activity of curcumin, there are limited data mainly related to the study of its pharmacokinetics and toxicity [125]. Curcumin is poorly absorbed following oral administration, as it is not hydrosoluble, in such a way that the majority of the ingested curcumin is excreted intact after passing through the digestive tract. The rest is metabolized at the intestine mucosa and liver rendering detectable curcumin plasma levels (0.41–1.75 μM after 1 h of oral dosing) when administered 4–8 g [126–128]. It has been shown that curcumin absorption is greatly improved by the concurrent oral administration of pipерин from black pepper [128]. In light of these observations, currently great efforts are being made to improve curcumin bioavailability and potentially study and exploit its therapeutic properties. This way novel curcumin formulations and complexes are being tested and include monocarboxyl analogs of curcumin [87], polymeric nanoparticle formulations of curcumin [92,129], and curcumin and phospholipids complexes [130]. On the other hand, curcumin administration has been shown to be fairly safe for humans even at high doses of 12 g/day [131]. Nevertheless, a recent study has pointed out the potential toxic activity of several components of turmeric accompanying curcumin from an in silico analysis [132], what deserves further in vivo studies.

5. Conclusions

Curcumin is a highly pleiotropic and multiactivity molecule that is capable of significantly affecting a wide variety of processes, ranging from the catalytic activity of antioxidant and proinflammatory enzymes to the expression of multiple genes related to inflammation and redox biology. Curcumin is a micronutrient that is closely related to cellular redox balance and thus to mitochondrial biology, making it the principal cell organelle for cellular redox control. Not surprisingly, the properties and activity of curcumin give rise to a network of molecular reactions that in every case and biological situation affect the mitochondria. Fat accumulation is a key process in many liver diseases, and various studies have shown that mitochondrial-related oxidative stress can be attenuated by specific dietary fat types, which in addition reduce blood lipids [133–136]. Therefore, the combined use of these dietary fats and curcumin would be an interesting approach to the treatment of liver pathologies. Specific targeted therapies and/or agents for mitochondrial disorders and related diseases or degenerative processes are extremely scarce, with many of them involving severe side effects [137]. On the other hand, despite the proven enormous therapeutic potential of curcumin and its safety for human use, it has not yet been approved for use in the treatment of any human disease. Therefore, future research into mitochondrial dysfunction-associated diseases, such as chronic liver disease, should focus on large, well-controlled human studies to fully develop the therapeutic potential of this micronutrient.

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