Oxidative Stress-induced liver damage and remodeling of the liver vasculature

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Abstract

As an organ critically important for targeting and clearing viruses, bacteria, and other foreign material, the liver operates via immune-tolerant, anti-inflammatory mechanisms indispensable to the immune response. Stress and stress-induced factors disrupt the homeostatic balance in the liver, inflicting tissue damage, injury, and remodeling. These factors include oxidative stress induced by viral infections, environmental toxins, drugs, alcohol, and diet. A recurrent theme seen amongst stressors common to multiple liver disease is the induction of mitochondrial dysfunction, increased ROS expression and depletion of ATP. Inflammatory signaling additionally exacerbates the condition, generating a pro-inflammatory, immunosuppressive microenvironment and activation of apoptotic and necrotic mechanisms that disrupt the integrity of liver morphology. These pathways initiate signaling pathways that significantly contribute to the development of liver steatosis, inflammation, fibrosis, cirrhosis, and liver cancers. In addition, hypoxia and oxidative stress directly enhance angiogenesis and lymphangiogenesis in chronic liver diseases. Late-stage consequences of these conditions often narrow the outcomes for liver transplantation or result in death. This review provides a detailed perspective on various stress-induced factors and the specific focus on role of oxidative stress in different liver diseases with special emphasis on different molecular mechanisms. It also highlights how resultant changes in the liver vasculature correlates with pathogenesis.

Keywords: Liver, vasculature, oxidative stress, liver injury, hepatic lymphatics.
Introduction

Stress induced health issues are one of the major challenges in modern society. Stress, which is defined by “conditions where an environmental demand exceeds the natural regulatory capacity of an organism, in particular situations that include unpredictability and uncontrollability” \(^1\) can alter the normal homeostasis of human physiological and psychological status \(^2,3\). Both experimental and clinical studies show that stress significantly impacts hepatic physiology and contributes to liver diseases. Further, it can also worsen the conditions of the patients with cardiovascular diseases, cancer and HIV/AIDS \(^2-4\).

Liver, the largest solid organ of the body, is the center of metabolism, detoxification and also a site for complex immunological activity controlled by diverse immune cells populations, as well as non-hematopoietic cells \(^5\). Liver receives the digested products, microbial agents and antigens from the body through the portal vein and they can elicit immune cell activation, and cause persistent and regulated inflammation \(^6\). Prolonged exposure to high-fat diet, alcohol, drugs and stress hormones causes chronic liver inflammation leading to chronic liver diseases \(^7\). However, under episodes of stress conditions, which can include physiological, psychological or environmental stressors, there can be a negative impact with long-term consequences on the patients with pre-existing conditions such as hepatitis B (HBV) \(^8\), hepatitis C (HCV) \(^9\), alcoholic hepatitis \(^10\), non-alcoholic steatohepatitis (NASH)\(^11\), oxidative stress or endoplasmic reticular stress (ER-stress) in hepatocellular carcinoma (HCC) patients\(^12,12,13\). In these stress mediated liver damages, both the hepatocytes as well as the non-parenchymal cells which are present in the liver vasculature are impacted \(^7\). In this current review, the principal contributing factors for stress induced damage in liver and the liver vasculature leading to progression of liver disease have been highlighted.
Liver anatomy, vasculature, and cellular distribution

The liver is a highly vascularized organ with a dense network of blood vessels, bile ducts and lymphatic vessels. The liver plays a critical role in transforming toxic substances of the body into a form which can be eliminated. Compared to the other organs of the body, liver has a high capacity to receive the cardiac output and at rest, it can receive 25% of cardiac output. It is also the first organ to receive nutrient rich blood from the intestines and participates in immune response. Liver is the unique organ of the body with the superimposition of two inlet networks, i.e., hepatic artery and the portal vein, running in parallel; and one outlet network composed of central veins, or hepatic veins. The hepatic artery and portal vein play the major role in maintaining the blood supply in the liver. Liver is divided into hexagonal hepatic lobules, which consist of the central vein, portal triads and liver resident cells. Portal triad is composed of portal vein, hepatic artery, and bile ducts. (Figure 1). The portal vein collects the blood from digestive tract, pancreas, spleen and then it contributes to approximately 75% of the liver’s blood supply, while the rest 25% is supplied by hepatic artery. The venous blood then mixes with oxygenated blood from hepatic artery and flow through the liver sinusoid, the microvascular bed of liver and the sinusoids send the blood to the central vein. LSECs are both morphologically and functionally different from the blood endothelial cells, as they form clustered fenestrations on sieve plates, form a discontinuous arrangement, lack the typical basement membrane and have the attenuated extracellular matrix with fibronectin as the major component. In adult human liver, the length of sinusoids is approximately 250 µm and its diameter varies between 7-15 µm. However, under normal physiological condition, the unique arrangement of the LSECs facilitate exchange of large molecules and pathogens between hepatocytes and blood.
Bile ducts carry the bile from the liver to the gallbladder in an opposite direction to the liver sinusoids\textsuperscript{20}. Along with the blood vasculature, the liver has dense lymphatic vasculature and the lymphatic system is critical for maintenance of body fluid homeostasis, inflammation, and immune response\textsuperscript{21, 22}. Recent studies in murine models show that the liver lymphatics are organized segmentally, and intrahepatic lymphatic vessels are populated majorly around the portal triad\textsuperscript{23}. Lymphatic capillaries, the thin-walled vessels composed of single layered lymphatic endothelial cells (LECs) collect the lymph, which is rich in cellular proteins, lipoproteins and lymphocytes, and drain into the collecting lymphatic vessels. Collecting lymphatic vessels, unlike the lymphatic capillaries, are covered by lymphatic muscle cells which can pump the lymph from the liver to the lymph nodes\textsuperscript{24, 21}.

Hepatocytes

In the adult human liver, 70-80% is occupied by parenchymal hepatocytes. Hepatocytes play crucial roles in metabolism, detoxification, innate immune regulation, protein synthesis and protein secretion in the circulation\textsuperscript{25, 26}. The main proteins secreted by the hepatocytes include α-fetoprotein, serum albumin, transferrin, plasminogen, fibrinogen, α-1-antitrypsin, C-reactive protein and blood clotting factors (except for f factor VIII)\textsuperscript{25}. The hepatocytes are the first site for initiation of bile acid (BA) synthesis, which is then transported to the bile canaliculi. BAs take part in the emulsification, digestion of dietary fats and removal of metabolic wastes\textsuperscript{25}. Bile is transported through the series of bile ductules to bile ducts and finally the hepatic bile is transferred to the gallbladder and stored there until being hormonally stimulated and released into intestines. However, when the BAs are retained into the liver due to the impairment of its secretion, it causes hepatocellular toxicity\textsuperscript{27}. Under pathological conditions, enhanced level of BAs, Deoxycholic acids and taurodeoxycholic acids in the liver induced the reactive oxygen species (ROS)
Stress Responses and Cellular Crosstalk in the Pathogenesis of Liver Disease Theme Issue

production, which in turn activate the downstream signaling of ERK1/2 and AKT pathways. Hepatocytes are the major targets of ROS mediated damage. High level of ROS has been shown to stimulate mitochondrial hypertonicity leading to release of cytochrome C and hepatocyte apoptosis.

Cholangiocytes

The three-dimensional network of bile duct, or biliary tree, are lined with the heterogeneous, highly dynamic population of epithelial cells or cholangiocytes and they represent only 3-5% of total liver cell population. However, cholangiocytes are responsible for up to 30% of total bile flow in humans, and the rest of 70% is produced from hepatocytes. Cholangiocytes are activated by several stimuli which include xenobiotic, cholestasis, ischemia, infections, oxidative stress and activated cholangiocytes have altered proliferation capacity, secrete variety of pro-fibrotic and pro-inflammatory factors.

Hepatic stellate cells (HSCs)

Hepatic stellate cells, which represent about 10% of the liver cells, are the liver specific fibroblasts which reside in the Space of Disse, the space between the LSECs and hepatocytes (Figure 1). In healthy liver, HSCs are the primary storage site and metabolism sites of vitamin A. In case of liver injury, the HSCs get activated, differentiated into myofibroblasts, produce extracellular matrix components (ECM) and cause fibrosis in the liver. Interestingly, activated HSCs also produce plethora of cytokines and chemokines, which include monocyte chemoattractant protein 1 (MCP-1), interleukin-6 (IL-6), TGF-β which enable leukocytes recruitment into the liver. Recruited leukocytes secrete the proinflammatory factors and elicit the immune response as well as contribute to further activation of HSCs. Under oxidative stress, ROS, activates the quiescent
HSCs and causes ECM production which in the long run contribute to fibrosis, cirrhosis, and HCC.

Kupffer Cells (KCs)

Kupffer cells (KCs) are the main liver resident macrophages and are present in the liver sinusoid and adherent to the LSECs of the sinusoidal wall (Figure 1). The KCs serves as the first line of defense against bacterial infection and play important roles in host immune response. Under pathological conditions, KCs are differentiated into M1-like (classical) or M2-like (alternative) macrophages. KCs are major sources of pro-inflammatory cytokines which can cause liver damage. In response to the stimuli, oxidized low-density lipoprotein (LDL) and lipopolysaccharide (LPS), the KCs and infiltrating macrophages in the liver, produce ROS through the activation of Nox2 which eventually stimulate the KCs to secrete the pro-inflammatory cytokines, e.g., TNF-α, IL-6, and IL-1β.

Liver sinusoidal endothelial cells (LSECs)

The highly specialized LSECs are specialized endothelial cells in the liver, that form the interface between the lumen of sinusoid and the liver hepatocytes and hepatic stellate cells. In contrast to the other endothelial cells, LSECs displays clusters of fenestrations which form the “sieve plates” and due to the lack of organized basement membrane, the LSECs form highly permeable membrane. In normal liver, LSECs, selectively control the diffusion of substrate between the blood and the space of Disse. The phenotypes of the LSECs are maintained by the vascular endothelial growth factor-A (VEGF-A) stimulation of nitric oxide (NO)-dependent or NO independent signaling. In healthy liver, LSECs keep the HSCs quiescent. HSCs are the major source of fibrillar collagens and other components of the liver scar. In diseases condition, the
damaged LSECs secrete proinflammatory cytokines, TGF-β and stimulate the HSCs and liver hepatocytes which in turn cause further liver damage \(^{7,34}\).

Liver lymphatic endothelial cells (LECs)

Liver is the largest lymph producing organ of the body and it has highly dense lymphatic \(^{46}\). The plasma component of the blood is filtered through the fenestrae of the LSECs and transferred to the Space of Disse. This fluid flows through the space of mall, enters the interstitial space of the portal triad and finally the lymphatic capillaries \(^{24}\). The lymphatic vessels are primarily located around the portal triad and drain the lymph to nearby draining lymph nodes outside the liver \(^{47}\). Inside the liver, the lymphatic vessels are composed of lymphatic endothelial cells (LECs), and LECs are identified by specific markers Lyve-1, Prox1, podoplanin (PDPN) and absence of αSMA-positive cells (smooth muscle cells/pericytes). It is noteworthy to mention that LSECs also express Lyve-1 and hepatocytes express Prox1 \(^{48}\). Thus, the co-existence of the above three markers identifies LECs. In normal healthy livers, LECs are required to maintain the homeostasis of fat metabolism as they can uptake the cholesterol through the scavenger receptor class B type I \(^{49,50}\). Several studies reported that, under pathological conditions, enhanced level of oxidative stress, which is associated with production of reactive oxygen species (ROS), increased the proliferation of LECs causes expansion of the lymphatic network or lymphangiogenesis \(^{24,51-53}\).

**Contribution of Stress and stress induced factors to liver injury**

This current review primarily focuses on the various aspects of oxidative stress and reactive oxygen species on liver diseases and impact on liver vasculature.

Oxidative stress (OS)
The liver is the primary detoxifying organ that metabolizes various compounds that produce free radicals. Liver homeostasis is threatened when the number of free radicals, including ROS, exceeds the endogenous antioxidant components. An excessive amount of ROS within hepatocytes can cause damage to the proteins, lipids, and DNA. The process results in structural and functional abnormalities in the liver that develop into various diseases such as HCC, chronic hepatitis, and fibrosis. The sections below provide a comprehensive review of the sources, consequences, and treatments of OS on liver disease and liver vasculature.

Sources of Reactive Oxygen Species (ROS)

The mitochondria and endoplasmic reticulum (ER) are the main sites of ROS formation within hepatocytes. Several extrinsic (alcohol consumption, drug overuse, environmental toxins, viruses, smoking) and intrinsic sources (obesity and insulin resistance) can promote ROS production in the liver. Under pathological conditions, liver inflammations or injury, multiple cells contribute to the production of ROS. ROS is a double-edged sword and different concentrations can also have different physiological impact on the surrounding vasculature. High concentrations of ROS over a long period are harmful to tissues, while short or low concentrations of ROS have been shown to promote neovascularization and also activate different signaling mechanisms. The mitochondrial transport chain transfers electrons and hydrogen through NADH dehydrogenase and ubiquinone cytochrome C reductase to produce water as a non-toxic byproduct. Instead of accepting two electrons, O2 only accepts one electron, reducing O2 to O2−, superoxide. Superoxide is then converted to hydrogen peroxide, H2O2, by superoxide dismutase. H2O2 is then converted to hydroxyl radicals (·OH) via the Fenton reaction. Additionally, O2− reacts with nitric oxide, NO, which generates another ROS known as peroxynitrite, ONOO−. The highly reactive superoxide, O2−, and ·OH radicals react with other molecules to gain or lose an electron.
to become stable. The affected molecule becomes a free radical and creates a cascade of free radicals which damage the hepatocyte. The non-radical ROS, such as hydrogen peroxide, H$_2$O$_2$, will also lead to cascades of free radicals. By creating radical and non-radical ROS, damage in hepatocytes occurs in the form of oxidative DNA damage, abnormal protein expression, and oxidative degeneration of lipids. In figure 2, different stimuli stimulating mitochondrial dysfunction and hepatocyte damage have been described (Figure 2).

**OS in inflammatory liver diseases**

In the next section, liver diseases with OS response, including viral hepatitis, liver cirrhosis, HCC, cholangiocarcinoma (CCA), alcoholic liver diseases (ALD), non-alcoholic fatty liver diseases (NAFLD), drug induced liver injury (DILI) will be discussed.

OS in Viral Hepatitis

One of the most common etiologies of chronic liver injury is the chronic infection with hepatitis B (HBV) and hepatitis C viruses (HCV). The main viral hepatitis is hepatitis A-E, along with which only hepatitis B, C, D and E are known to cause chronic liver diseases. In case of HCC, it has been reported that, 80% of HCC are associated with chronic infection with HBV and HCV. HCV, among all viral hepatitis, is responsible for liver fibrosis, HCC, steatosis, and liver failure. In HCV patients, an elevated level of oxidative stress, high levels of ROS and reactive nitrogen species (RNS) have been reported. The NS3 protein of HCV activates Nox 2 protein (the major component of redox system), on the phagocytes, and causes apoptosis and dysfunction of T cells and natural killer cells, and as a result increases ROS production by the surrounding neighboring cells. The HCV core protein plays a critical role in the development of chronic HCV and HCC by activating TNFR, PKR, and STAT3 mediated pathways. Higher level of ROS causes the
damage of liver cells and vasculature. HBV also infect KC that signal macrophages to produce proinflammatory cytokines such as Interleukin-1 (IL-1), IL-6, C-X-C Motif Chemokine Ligand 8 (CXCL-8), and TNF-α. These cytokines damage mitochondrial cytochrome oxidase inhibiting the electron transport chain, increasing ROS levels, and inducing HCC. Furthermore, HCV-related HCC patients have more markers of OS, such as 8’-hydroxy-2’-deoxy guanosine, 8-OHdG, and reactive oxygen metabolites, in their serum than HBV-related HCC patients, indicating more significant levels of OS in HCV infection. Chronic HBV and HCV infection are also associated with elevated level of bivalent irons accumulated in the liver which also cause the production of ROS, and hydroxyl free radicals. ROS also acts as an inducer of VEGF production. In chronic HCV patients, there is also high level of VEGF, which is one of the major angiogenic factors. The HBV infection induces the mitochondrial ROS accumulation in hepatocytes which causes the sustained activation of IL-6/STAT3 pathway and increase in production of downstream factor Vascular Endothelial Growth Factor (VEGF). Altogether, the HBV induced OS promotes the hepatocarcinogenesis. The HBx protein, a regulatory protein produced by HBV, promotes activation and stabilization of Hypoxia-inducible factor-α (HIF-1α). Activated HIF-1α, which is the upstream regulator of VEGF, enhanced the transcription of VEGF and finally promote the angiogenesis.

OS in liver cirrhosis and fibrosis

Hepatic stellate cells (HSC) and KCs are related to the development of liver cirrhosis and fibrosis. OS promotes the activation of HSCs, which can produce ECM and contribute to the development of liver fibrosis, a significant risk factor for HCC. ROS and O₂⁻ activate HSC and damage hepatocytes while also triggering the activation of NF-κB, which regulates genes involved in cell transformation, proliferation, and angiogenesis. NF-κB creates a positive feedback loop, further
increasing nitrogen monoxide (NO) and ROS production and forming oxidized low-density lipoprotein (OxLDL), exacerbating hepatocyte damage \(^{68}\). In addition, KCs are constantly activated in liver fibrosis and cirrhosis, producing large amounts of extracellular ROS that cause hepatocyte necrosis \(^{87}\).

OS in Liver Cancers: HCC and CCA

In chronic hepatitis patients, an increased amount of ROS-mediated DNA damage, accumulation of mutations, chromosomal rearrangements and genomic instability in hepatocytes can cause extensive cellular and molecular alterations in the hepatocytes and its microenvironment, that can lead to cirrhosis and eventually HCC\(^{68, 88}\). In addition to DNA damage, OS can activate various signaling pathways that promote the growth and survival of cancer cells. ROS can activate the NF-κB pathway, enabling the expression of genes involved in cell proliferation, angiogenesis, and apoptosis resistance\(^{89}\). ROS can also activate the MAPK/ERK pathway, which is involved in cell growth and survival \(^{90}\). OS can also stimulate the production of proinflammatory cytokines and chemokines, attracting immune cells and promoting inflammation in the liver. Chronic inflammation can also contribute to the development of HCC by promoting the proliferation and survival of cancer cells \(^{88}\).

CCA is a heterogeneous group of malignancies in the biliary tree with a dismal 5-year survival rate after metastasis to distal organs \(^{22, 91}\). In fact, the CCA metastasis to the draining lymph nodes (LNs) with enhanced level of both intra-and peri-tumoral lymphangiogenesis is the primary prognostic factor of the aggressiveness of this disease \(^{22}\). In both human tissues and murine models of iCCA, mitochondrial dysfunction and oxidative stress in the tumor micro-environment, have been shown to promote the accumulation of ROS and infiltration of TNF-α producing macrophages, which in turn creates a favorable environment for the proliferation of biliary cells.
In cholestatic liver diseases, high level of BAs, through its cognate receptor Takeda G protein-coupled receptor 5 (TGR5) on cholangiocytes, induces increased production of ROS, activates cSrc mediated epidermal growth factor receptor (EGFR) transactivation and subsequent Erk1/2 phosphorylation. As a result of this, the cellular proliferation is increased. Under inflammatory conditions, inflamed LECs secrete high levels of chemokine CXCL5, which binds to its receptor CXCR2 on CCA cells, alter cellular metabolism, enhances production of mitochondrial ROS, and promotes lymphangiogenesis and tumor inducing pathways in CCA.

OS in Alcoholic liver disease (ALD) and Non-alcoholic fatty liver disease (NAFLD)

ALD is a predominant form of chronic liver disease globally, inflicted by excessive alcohol consumption that varies in quantities between individuals. ALD initiation is marked by alcoholic fatty liver (AFL), defined by the accumulation of triglycerides in hepatocytes known as hepatic steatosis. The condition can then progress to inflammation and fibrosis, driving the eventual development of cirrhosis, liver failure, and HCC. A chain of events is derived from the hepatotoxicity of ethanol metabolism, producing the toxic and carcinogenic product acetaldehyde via the oxidation of ethanol through the alcohol dehydrogenase (ADH) pathway. Cytochrome P450 2E1 (CYP2E1), an enzyme in the endoplasmic reticulum (ER) and mitochondria of hepatocytes, additionally bolsters these products by metabolizing alcohol to acetaldehyde in the presence of oxygen and NADPH. Stimulated by chronic alcohol consumption, CYP2E1-led alcohol metabolism is another pathway for alcohol oxidation. Acetaldehyde promotes the production of neoantigens, subsequently causing cirrhosis of the liver, increase in oxidative stress, altered DNA methylation, and a reduction of retinoic acid thereby instigating hepatocarcinogenesis. Ethanol and acetaldehyde thus have a deleterious effect on hepatocytes by generating ROS and damaging intestinal mucosal barrier. Cellular oxidative stress caused by an excess of free...
radicals and the deficiency of glutathione, vitamin E, and phosphatidylcholine may be the primary factors behind the progression of alcoholic liver disease. Neoantigens from acetaldehyde may also propagate inflammation by cytokine and chemokine induction that is reported in individuals with acute alcoholic hepatitis. TNF-α and IL-6 are increased after chronic alcohol consumption following NF-κB activation, instigated by toll-like receptor 4 (TLR4) sensing lipopolysaccharides (LPS) 102.

OS also plays a predominant role in exacerbation of NAFLD, one of the leading causes of chronic liver disease worldwide, with a prevalence of 25% globally 103. Without alcohol or drugs implicated as causal factors, primary risk factors for disease progression include age, increasing BMI, high-lipid diets, and diabetes 104. The presence and stage of fibrosis is the foremost predictor of overall and disease-specific mortality, with liver-related mortality increasing exponentially with each progressive fibrosis stage 105. The chief cause of death for NAFLD patients is cardiovascular disease (40%), with recent studies suggesting NAFLD may directly heighten risk of heart disease 106. Polyunsaturated fats (PUFAs) have shown to exhibit important roles in NAFLD through pro-inflammatory and anti-inflammatory effects dependent on their structure 107. A high ratio of pro-inflammatory promoting n-6 PUFAs to anti-inflammatory n-3 PUFAs is correlated with NAFLD and non-alcoholic steatohepatitis (NASH), showing once again how inflammatory microenvironments induced promote liver disease progression 108. Innate immunity, determined by TLR signaling manipulation, inflammasome activation, macrophage activation, increase in pro-inflammatory cytokines and chemokines, and decreased immune cell recruitment (natural killer T-cells), can have major involvement in the progression to severe liver diseases like HCC 109-112. Additional causal factors include the development of lipotoxic lipids from saturated free fatty acid accumulation, which induces mitochondrial dysfunction, oxidative stress, and ROS much like
previously discussed \(^{113}\). Hepatocellular injury and death, triggered by autophagy, apoptosis, necroptosis, and pyroptosis, is also pivotal to NAFLD and supplement the progression towards chronic liver disease \(^{114-116}\). Beginning with NAFL, followed by NASH, fibrosis, cirrhosis, and finally liver cancers like hepatocellular carcinoma, the general progression from a healthy liver to chronic liver diseases can primarily be combatted by weight loss, with 10% weight loss accounting for nearly 50% of patients experiencing fibrosis regression \(^{117}\). Other management methods include monitoring blood pressure, lipid intake, and diabetes control.

Drug-Induced Liver Injury (DILI) and OS

DILI is the foremost instigator of acute liver failure, with causal factors such as antimicrobials, herbal agents, dietary supplements, cardiovascular drugs, central nervous system agents, antineoplastic drugs, and analgesics \(^{118,119}\). Intrinsic DILI categorizes the predictable dose-dependent sources of DILI, often associated with the drug’s toxicity and a quick onset of symptoms \(^{120}\). The analgesic Acetaminophen is often a cause of intrinsic hepatotoxicity, generation of oxidative stress, mitochondrial dysfunction, and endoplasmic reticulum (ER) stress, which can eventually cause cell death \(^{119}\). Table 2\(^{121-157}\) compiles an array of drugs known to induce stress and lead to liver injury. By keeping record of common drugs with hepatotoxic implications, prediction, prevention, and mitigation of liver injury can be supplemented. It is important to continue investigation into the hepatotoxic qualities of drugs that are still in development, for effective clinical management of liver damage and disease.

The principal drug or its associated metabolites, metabolized by the cytochrome P450 (CYP450) family that carries out oxidative phase-I drug metabolism are shown to initiate DILI by direct cell
stress, targeting mitochondrial function, or catalyzing certain immune functions. Metabolites from phase I and conjugative phase II metabolism induce cellular stress through direct binding of cell structures, such as enzymes, lipids, and nucleic acids, or by glutathione depletion. Mitochondrial malfunction occurs by the metabolite-navigated uncoupling of the mitochondrial electron transport chain, depriving cells of major portions of ATP, increasing ROS, inhibiting \( \beta \)-oxidation, altering mitochondrial DNA composition and replication, or inducing the mitochondrial permeability transition pore (MPT) to open on the inner mitochondrial membrane. Mitochondrial dysfunction can be propelled intrinsically, during which severe cellular stress activates MPT by activation of pro-apoptotic and inhibition of anti-apoptotic proteins of the Bcl-2 family following stimulation of lysosomal permeabilization, the endoplasmic reticulum pathway, or c-jun N-terminal kinase (JNK). Mitochondrial dysfunction also follows extrinsic inflammatory responses instigated by the innate immune system, which predispose the liver to be more vulnerable to the lethality of TNF-\( \alpha \), Fas ligand, and interferon gamma. The consequent disruption of the proton gradient driving most ATP production in the mitochondria leads to necrotic or apoptotic cell death in the liver.

**Impact of OS on liver vasculature**

Oxidative stress and Angiogenesis in the liver

Along with the damaging effect on liver cells, oxidative stress also positively regulates angiogenesis and lymphangiogenesis in several liver pathologies, including HCC and CCA. In the case of pathological angiogenesis, the demand for excess oxygen in surrounding tissues result in hypoxia/re-oxygenation cycle. Under hypoxic condition, the insufficiency of oxygen supply to the hepatocytes and other liver tissue causes pathological changes, leading to liver damage. The liver is susceptible to hypoxic injury due to its central location in the circulatory system and high
metabolic demand. During hypoxia, the decreased oxygen supply leads to a disruption of oxidative phosphorylation, which results in decreased ATP production and an increase in ROS production. These changes can lead to mitochondrial dysfunction, OS, and inflammation, ultimately resulting in cell damage and death. Hypoxia-induced liver damage has been linked to the activation of hypoxia-inducible factor, HIFs, a family of transcription factors that play a critical role in the cellular response to hypoxia. HIFs promote the expression of genes involved in angiogenesis, such as VEGF; metabolism, and cell survival, that contribute to the development and progression of liver disease. HIFs have been implicated in the development and progression of HCC, as they promote angiogenesis and tumor growth. In particular, the expression of VEGF is vital in liver angiogenesis, as it promotes the formation of new blood vessels from pre-existing vessels. VEGF stimulates endothelial cell proliferation, migration, and survival, leading to new blood vessel formation. By promoting angiogenic genes, such as VEGF, HIFs give rise to the development of liver fibrosis, cirrhosis, and metastasis of tumors in the liver. HCC is one of the most hypoxic malignancies. HCCs, like many other cancer cells, can grow in low oxygen levels or hypoxic environments. To survive and proliferate, HCC cells adapt to hypoxia through a concerted transcriptional response that HIFs regulate. Hypoxia-induced liver damage can have various consequences, depending on the severity and duration of the hypoxia. In mild cases, hypoxia may lead to a transient increase in liver enzymes, such as alanine aminotransferase (ALT), and aspartate aminotransferase (AST), indicating liver injury. In more severe cases, hypoxia can lead to acute liver failure, characterized by a rapid onset of liver dysfunction, which can be life-threatening. Hypoxia can also contribute to the development and progression of liver diseases, such as NAFLD, and cirrhosis. Chronic hypoxia can result in the accumulation of fat in the liver, which can progress to inflammation and fibrosis, ultimately leading to HCC.
Oxidative stress and Lymphangiogenesis

Several lines of evidence suggest that dysfunctional lymphatics is associated with inflammatory liver diseases. In the postnatal liver, the lymphatic system remains quiescent. However, under pathological conditions, like chronic hepatitis, liver fibrosis, and CCA, higher level of lymphangiogenesis have been reported. Although LECs are the cornerstone of new lymphatic vessel formations, but other cells, which include macrophages, dendritic cells, kupffer cells, T-cells, and B-cells contribute to lymphangiogenesis. One major cause of enhanced level of lymphangiogenesis is tissue inflammation. Under inflammatory conditions, surrounding cells in lymphatics vasculature produce VEGF, including VEGF-C and VEGF-D, the major growth factors for growth of lymphatic vasculature. In inflamed skin, a profound infiltration of CD11b+/Gr-1+ in the draining lymph nodes and the inflamed skin has been reported which in turn secrete VEGF-C, D and A and contribute to the increase in lymphangiogenesis. In cholestatic liver diseases, primary sclerosing cholangitis (PSC), a progressive biliary inflammation is associated with increased level of BA in the liver. Our recent work revealed that, in MDR2-/- knock out mice, an in vivo model of sclerosing cholangitis, elevated levels of conjugated BAs were associated with increased lymphangiogenesis. Conjugated BAs, taurocholic acid and chenodeoxycholic acids induced oxidative stress with enhanced levels of ROS in LECs. The heightened level of ROS-mediated oxidative stress in LECs activate the redox sensitive kinase p90RSK which, via the post-translational modification of the transcription factor Prox1 cause the transcriptional activation of VEGFR3 and promotes lymphangiogenesis. Thus, inflammation, ROS and oxidative stress induced LEC proliferation and lymphangiogenesis are closely associated with progression of liver disease.

Endogenous and dietary antioxidants: tools for rescuing from the OS in liver diseases.
The impact of OS on the development and progress of stress related liver pathologies highlighted the importance of promoting the level of endogenous antioxidant levels and/or supplementation with dietary antioxidants. The cells in the liver have their own defense mechanism to produce antioxidants enzymatically or nonenzymatically to neutralize the cellular ROS. The important enzymatic systems involved in this redox control mechanism are super-oxide dismutase (SOD), catalase (CAT), reductase (GSH-Prx or Gpx), glutathione peroxidase etc. The antioxidant enzymes are in different cellular and subcellular locations. For example, SOD has two isoforms, the SOD-1 which is a cytosolic (Cu/Zn–SOD) isoform and SOD-2, the mitochondrial (Mn-SOD) isoform. SODs act the first line of defense in the antioxidant mechanism in hepatocytes by reducing the superoxide radicals to \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \). The \( \text{H}_2\text{O}_2 \) is then degraded by other detoxifying enzymes, CAT, mitochondrial glutathione peroxidases (Gpx1,4), s hydroperoxides (PrxIII, Trx2). Along with the enzymatic antioxidant systems, the non-enzymatic molecules, like, glutathione, retinol (Vitamin A), ascorbic acid (Vitamin C), tocoferol (Vitamin E) scavenge the free radicals produced by the cells. Another regulatory mechanism in the antioxidant defense is the transcriptional regulation of the antioxidant genes, regulated by nuclear factor E2-related factor 2 (Nrf2). The Nrf2 controls an array of antioxidant genes. Thus, boosting the Nrf2 machinery with exogenous agents is an important strategy to control the redox balance in the cells. Dietary supplements with anti-oxidative properties are considered as a strategy for oxidative stress induced liver diseases. Dietary cocoa in chocolate, rich in antioxidants is effectively used for the amelioration of NAFLD. Ginger is another commonly used dietary antioxidant which is effective in prevention of stress induced liver diseases. Lemon juice, green tree extracts, quercetin and resveratrol from plant extracts have proven to have antioxidant property.
and are used as potential compounds for the treatment of stress induced liver diseases. In table 3, a list of potential antioxidants in the context of OS induced liver injury has been added.

**Conclusion and Future Approach:**

The dense vascularization of the hepatic lobules by blood vessels, bile ducts, and lymphatic vessels commit the liver to a multitude of tasks ranging from fluid homeostasis, immune surveillance, detoxification, bile production, and other functions proffered by its morphology. Maintaining a healthy liver anatomy is crucial for a high quality of life. Since OS and ROS play critical role in liver pathologies and liver vasculature, in this current review, the role of oxidative stress induce liver and liver vasculature damage were discussed in detail to elucidate what sources impair these requisite functions and how they do so. The diverse pathways discussed that lead to liver damage, including oxidative stress, viruses, environmental toxins, drugs, alcohol, and high-fat diet, all converge at one point—increased mitochondrial ROS output. The molecular pathways induced by ROS include NF-kB, MAP/ERK, and JNK/c-Jun and these alter the complex microarchitecture constructed by parenchymal hepatocytes, cholangiocytes, LSECs, HSCs, and LECs of the liver, inflicting cell death and altered cellular secretions that further catalyze liver damage. Subsequently, angiogenesis, lymphangiogenesis, inflammation, and increased tumor cell invasion occurs. Once progressed to this point of morphological disturbance, prognoses often develop into liver fibrosis, cirrhosis, and liver cancers. Further research delving into the molecular pathways stimulated by ROS and pharmaceutical inhibitors that may mitigate the liver and liver vasculature damage caused by these pathways warrant attention. Additional elaboration of drugs that are focused on alleviation of oxidative stress mediated damage, thus is of profound importance.
Through these efforts, the dismal outcomes associated with accumulated liver damage over years may be alleviated.

**Author contributions:**

S.C., P.B., N.G. designed the article. P.B., N.G., V.C. did the literature review and wrote the article. S.C. provided intellectual input and supervised. All authors have critically reviewed and edited this manuscript. All authors have read and agreed to the published version of the manuscript.
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[70] Lim HK, Jeffrey GP, Ramm GA, Soekmadji C: Pathogenesis of Viral Hepatitis-Induced Chronic Liver Disease: Role of Extracellular Vesicles. Front Cell Infect Microbiol 2020, 10:587628.


Figure legends:

**Figure 1:** Morphology of the hepatic lobule. The functional unit of the liver, the hepatic lobule, has a hexagonal structure with a central vein. Sinusoid vessels and rows of hepatocytes radiate outwards to the portal triad—composed of the bile duct, portal vein, and hepatic artery. Looking closer at the sinusoid, the walls are composed of LSECS with adherent and macrophagic KCs populating the lumen. Just on the periphery are the fibroblast stellate cells residing in the Space of Disse between the LSECs and liver hepatocytes. (This schematic was created with BioRender.com; Toronto, Canada). Last accessed on March 1st, 2023.

**Figure 2:** Induction of liver damage by mitochondrial dysfunction. Through an array of stimuli, including drugs, alcohol, viruses, free fatty acids (FFAs), stress hormones, and oxidative stress, mitochondrial functionalities can be impaired. This dysfunction may be the result of uncoupling of the electron transport chain (ETC), membrane permeability transition (MPT), or alteration of mitochondrial DNA (mtDNA). Consequently, ATP is depleted, B-fatty acid oxidation is down, and reactive oxygen species (ROS) accumulation increases. This then stimulates several signaling axes, including NF-κB, MAPK/ERK, and JNK/c-Jun. All lead to liver damage. At the bottom, the progression of a healthy liver to a liver with hepatocellular carcinoma (HCC) is depicted to portray the how constant damage amassed manifests over time. (This schematic was created with BioRender.com; Toronto, Canada). Last accessed on March 1st, 2023.
# Table 1: Cellular sources of ROS:

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Disease conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune cells</td>
<td>In cancer patients, Hypoxic condition, elevated level of TNFα, chemotherapeutics induce ROS production[^57]</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>Chronic hepatic inflammation, ischemia/reperfusion injury[^58], hypoxia induced hepatocyte injury[^58, 59]</td>
</tr>
<tr>
<td>Kuffer cells</td>
<td>Ischemia/reperfusion injury in rat livers[^60]</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Inflammation induced liver damage[^61], CCL4 induced liver damage[^62]</td>
</tr>
<tr>
<td>Hepatic stellate cells</td>
<td>Response to Oxidative Stress induce Liver Injury and Fibrosis[^63]</td>
</tr>
<tr>
<td>Natural Killer cells</td>
<td>Activity of NADPH oxidases generates superoxide anions[^64], Cytokine release induce chronic inflammatory conditions[^65]</td>
</tr>
<tr>
<td>Liver sinusoidal</td>
<td>Inflammatory Cytokines, Oxidative Stress[^66], Development of alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH)[^67]</td>
</tr>
<tr>
<td>endothelial cells</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Examples of hepatotoxic drug that may induce liver injury.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Usage</th>
<th>Suggested Mechanisms of Injury</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acarbose</td>
<td>Diabetes</td>
<td>Idiosyncratic</td>
<td>121, 122</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>Anti-arrhythmic</td>
<td>Kupffer cell activation, mitochondrial stress, and oxidative stress</td>
<td>123, 124</td>
</tr>
<tr>
<td>Bosentan</td>
<td>Pulmonary Hypertension</td>
<td>Bile salt export pump inhibition</td>
<td>125, 126</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Analgesic</td>
<td>Increased intracellular ROS output and lysosomal dysfunction suppress autophagy</td>
<td>127, 128</td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>Antibiotic</td>
<td>Production of the cytotoxic metabolite 5′-hydroxymethyl flucloxacillin by CYP3A4, CYP3A7 and CYP2C9 enzymes</td>
<td>129, 130</td>
</tr>
<tr>
<td>Flutamide</td>
<td>Anti-androgen</td>
<td>Heme oxygenase-1 inhibition regulated by nuclear factor erythroid 2-related factor 2 (Nrf2)</td>
<td>131, 132</td>
</tr>
<tr>
<td>Halothane</td>
<td>Anaesthetic</td>
<td>Conversion of halothane to trifluoroacetyl chloride by cytochrome P450, forming trifluoroacetylated liver proteins</td>
<td>133, 134</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>Antibiotic</td>
<td>Increased intracellular ROS output and weakened antioxidant capacity, endoplasmic reticulum stress, cell apoptosis, and liver injury; Nrf2 activation</td>
<td>135, 136</td>
</tr>
<tr>
<td>Metformin</td>
<td>Diabetes control</td>
<td>Autophagy and immune cell activation, liver-specific regulatory T cells, and expression of iNOS</td>
<td>137, 138</td>
</tr>
<tr>
<td>Minocycline</td>
<td>Antibiotic</td>
<td>Neoantigen formation followed by activation of CD8 T-lymphocytes with nonselective antigen receptors, poor immune regulatory mechanisms</td>
<td>139, 140</td>
</tr>
<tr>
<td>Drug</td>
<td>Class</td>
<td>Effects</td>
<td>References</td>
</tr>
<tr>
<td>--------------------</td>
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<td>---------------------------------------------------------------------------------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Nefazodone</td>
<td>Antidepressant</td>
<td>Increased ROS output, interference with OXPHOS enzymatic activities, and lower antioxidant defenses</td>
<td>141, 142</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>Antibiotic</td>
<td>Free radical production from nitro-reductive metabolism, suspected linkage with HLA-DR6 and DR2</td>
<td>143, 144</td>
</tr>
<tr>
<td>Periheziline</td>
<td>Antianginal</td>
<td>p38 and JNK signaling pathway activation, leading to endoplasmic reticulum stress</td>
<td>145, 146</td>
</tr>
<tr>
<td>Propylthiouracil</td>
<td>Antithyroid</td>
<td>Reactive metabolite production during myeloperoxidase action in neutrophils</td>
<td>147, 148</td>
</tr>
<tr>
<td>Sulindac</td>
<td>Analgesic</td>
<td>Increased ROS output leads to cytotoxic interaction between SLD and TNF-α, activating caspase 3/7</td>
<td>149, 150</td>
</tr>
<tr>
<td>Tacrine</td>
<td>Alzheimer’s Disease</td>
<td>Increased ROS output and glutathione depletion</td>
<td>151, 152</td>
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<tr>
<td>Tolcapone</td>
<td>Parkinson’s Disease</td>
<td>Mitochondrial uncoupling of oxidative phosphorylation</td>
<td>153, 154</td>
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<tr>
<td>Trovafloxacin</td>
<td>Antibiotic</td>
<td>Induction of proinflammatory M1-like macrophages, the interferon type I pathway, cytokines, and the apoptosis pathway</td>
<td>155, 156</td>
</tr>
<tr>
<td>Zileutin</td>
<td>Asthma</td>
<td>Patient-by-patient differences in cytochrome P450 metabolism, glutathione-conducted detoxification, and signaling of the farnesoid X receptor</td>
<td>157</td>
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</tbody>
</table>
Table 3: List of potential antioxidants for liver diseases in human or rodents

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>Potential mechanisms and effect on liver diseases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin E</td>
<td>Improve the lipid metabolism and reduced the activation of HSC in NAFLD</td>
<td>187</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Alters the PARPα dependent fatty acid β-oxidation, reduced the high fat diet induced obesity in NAFLD, improved the glucose metabolism</td>
<td>187, 188</td>
</tr>
<tr>
<td>Caffeine</td>
<td>Increase Nrf2 levels, reduced the NF-κβ activation and inflammatory cytokine level, reduced the oxidative stress in NASH rat model</td>
<td>189</td>
</tr>
<tr>
<td>Metformin</td>
<td>Increase the anti-oxidative mechanism by NRF2, increased the expression of Heme-oxygenase-1 gene, reduced the oxidative stress in NAFLD, nonalcoholic steatohepatitis (NASH) and HCC</td>
<td>190</td>
</tr>
<tr>
<td>Hesperetin</td>
<td>Reduced hepatic oxidative stress and inflammation via PI3K/AKT-Nrf2-ARE pathway in NAFLD rat model</td>
<td>191</td>
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</tbody>
</table>