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Synergistic Hepatoprotection: Molecular Mechanisms And Therapeutic Potential Of Picrorhiza Kurroa And Piper Longum In Liver Disease Management

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Abstract

Background: A wide variety of liver diseases, including Drug-induced liver injury (DILI) and Nonalcoholic Fatty Liver Disease (NAFLD), are prevalent all over the World. Historically used as combinations for their multi-targeted effects has yielded unknown appositional effects between Picrorhiza Kurroa and Piper Longum in providing hepatoprotection. This Review aims to examine these possible synergistic effects.

Mechanisms: P. kurroa has the capacity to protect cells from damage by acting on the Nrf2/ARE antioxidant pathway; inhibiting the inflammatory response mediated by NF-kB, as well as down-regulating TGF-beta-mediated fibrogenesis. The main drawback of this is its rapid metabolism which limits clinical efficacy. P. longum is a bioenhancer due to its alkaloid, piperine (or piperine derivative), which also inhibits the CYP450 isoenzymes and P-glycoprotein efflux pumps, thereby increasing the systemic bioavailability of picrosides while adding an antioxidant and anti-inflammatory activity.

Conclusion: All preclinical studies demonstrate that the use of these herbs as a botanical formulation and herbal supplement produce superior biochemical and histological results compared to using individual herbs alone. Due to the abundance of compelling evidence from experimental research, clinical usage is limited by the lack of standardized formulations and large random controlled trials. Future studies should emphasize the validation of the mechanisms through omics-based technology and investigate delivery of these herbs using nanotechnology, as well as provide a rigorous evaluation of the synergy of the botanical formulation in clinical practice for the prevention and treatment of chronic liver disease.

Keywords: Picrorhiza kurroa; Piper longum; Hepatoprotection; Synergism; Bioavailability enhancer; Piperine; Picrosides; Oxidative stress; NAFLD; Drug-induced liver injury (DILI).

Introduction

Liver disease accounts for the vast majority of worldwide illness and death. It is a leading cause of illness and death globally, increasing demand for health care costs around the globe. Chronic liver disease and cirrhosis has been rated as one of the top five causes of worldwide death. Non-Alcoholic Fatty Liver Disease (NAFLD) has rapidly become the most commonly diagnosed liver issue as a consequence of the increasing rates of obesity and metabolic syndrome (1), (2). Other major contributors include drug-induced liver damage/ injury, Hepatitis B and C, as well as alcohol-related liver diseases. These three areas are still

presenting very significant clinical challenges in many parts of the world, especially developing countries, in which advanced health care resources are usually constrained or lacking completely (3); (4); (5).

Despite considerable advancements in contemporary medicine, we currently have inadequate options for hepatoprotective therapies in a variety of clinical situations. The majority of current treatment protocols for liver disease are limited to specific types of liver problems and are primarily supportive, meaning that there is little chance of reversing the hepatocellular damage that has already occurred (6). Most synthetic medications used for treating liver diseases focus on only one aspect of the disease (inflammatory process or oxidative stress); however, liver disorders are therefore complicated because they involve multiple causes and aspects of injury and damage that may arise from metabolic dysregulation, oxidative stress, inflammation, and apoptosis (7). Additionally, long-term therapy for liver diseases can result in side effects, high price, and low compliance among patients, which is leading to increased interest in alternative therapies that can be obtained from natural resources and have fewer adverse effects The herb Piper longum (Pippali) is well-documented in Ayurvedic medicine as one that helps aid digestion and metabolism. The main active ingredient, Piperine, is known to have antioxidant, anti-inflammatory and immunomodulatory effects. Additionally, Piperine has been shown to boost antioxidant activity in the liver and lower lipid peroxidation(8); (9). The long history of both herbs Picrorhiza kurroa and Piper longum have been used in ethnopharmacological medicine provides a solid scientific basis to do further studies on their possible hepatoprotective effects.

Oxidative stress, inflammatory cytokines, mitochondrial dysfunction, and the dysregulation of apoptosis and fibrosis all contribute to the molecular pathogenesis of hepatobiliary diseases (10). Therefore, a multi-faceted approach will need to target the various mechanisms involved in hepatitis, which will ultimately lead to a successful therapeutic outcome.

Picrorhiza kurroa and Piper longum are combined in a pharmacologically appropriate manner because of their potential to synergistically enhance each other's efficacy; the hepatoprotective effect of P. kurroa and the powerful antioxidant properties of P. longum have been demonstrated (11); (12). This approach also reflects Ayurvedic medicine, which utilizes the combination of herbs to provide not only additive therapeutic effects but also to increase their absorption and reduce their toxicity.

This review aims to present the evidence supporting the synergistic hepatoprotective effects of Picrorhiza kurroa and P. longum and to describe the molecular mechanisms through which these compounds may exert their actions. We compiled and evaluated current research studies from both in vivo and in vitro experimental models of chemically-induced hepatotoxicity and have concentrated on oxidative stress and inflammation-related pathways and hepatoprotection. The integration of traditional knowledge and contemporary pharmacological research will help spotlight the therapeutic significance of this herbal combination and assist in developing future research opportunities related to its application in the management of liver disease.

2. Botanical Profile and Phytochemistry

2.1 Picrorhiza kurroa

Picrorhiza kurroa, also known as Kutki, is a member of the Plantaginaceae family. It grows on the high slopes of the Himalayas at high altitudes of 3,000 to 5,000 metres in India, Nepal, Bhutan and Tibet, where it prefers wet, cool environments. Traditional medicine uses Picrorhiza kurroa as a bitter tonic to treat liver diseases like jaundice and hepatitis, and for other ailments such as fever, asthma, dyspepsia and inflammatory problems. Ayurveda classifies Picrorhiza kurroa under "Yakriti-uttejaka", a term used to describe a substance that stimulates liver detoxification and bile production. The liver-protective properties of Picrorhiza kurroa are largely attributed to iridoid glycosides, collectively called kutkin (i.e., picroside I, picroside II, kutkoside), as well as apocynin and androsin, which have demonstrated antioxidant, anti-

inflammatory, and protect against prevalent oxidative stress, and regulate inflammatory mediators involved with the liver injuries.

Standardization and quality control parameters

The quality of P. kurroa extracts varies due to different geographic locations of harvest, time of year harvested, as well as processing methods. To provide consistency in the quality of P.kurroa, standardized parameters must be established for all harvests. Generally, standards for P. kurroa will include macroscopic and microscopic quality control parameters, chemical compositions (physicochemical constants), chromatographic fingerprints, and quantitative analysis of the active compounds (picroside) using HPLC or HPTLC techniques. The most common method to provide a standard for P.kurroa is through the use of markers based on two active constituents (picroside I and kutkoside) derived from the roots of the plant (13).

2.2 Piper longum

Piper longum L., of family Piperaceae, is a fruit bearing thin aromatic climber with fruits having catkin-like spikes. It is commonly found in tropical and subtropical regions of India, Sri Lanka, Indonesia, and Southeast Asia, normally growing in moist, shaded habitats. The dried piper fruits (long pepper) are used in ayurvedic medicine system (14).

P. longum colloquially known as Pippali is used as a digestive stimulant, carminative, immunomodulator, and rejuvenating agent in traditional ayurvedic medicine system. It is used as a key ingredient in several classical formulations such as Trikatu, and is typically believed to enhance drug absorption and metabolic efficiency. It is used as adjunct therapy in liver and metabolic disorders to improve the efficacy of co-administered herbs or drugs (15); (16).

P. longum exerts its pharmacological activities owing to alkaloids present in it specifically piperine which is the most abundant and well studied chemical constituent of Pippali. Apart from piperine, other bioactive compounds present in Pippalli include piperlongumine, piperlonguminine, various related alkaloids, and volatile oils. These phytochemicals' have antioxidant, anti-inflammatory, hepatoprotective, and bioenhancing properties, contributing to the therapeutic versatility of the plant (17); (18).

Chemical characterization of P. longum is typically performed using chromatographic and spectroscopic techniques such as HPLC, GC–MS, LC–MS/MS, and NMR. Piperine is commonly employed as a chemical marker for standardization. Advanced metabolomic approaches are increasingly being used to capture the complex phytochemical profile and ensure quality assurance of herbal preparations (19).

2.3 Synergistic Phytochemical Interactions

The bioavailability of several drugs and phytoconstituents can be enhanced through inhibiting drug metabolizing enzymes, altering intestinal tract permeability and inhibiting efflux transporters (e.g., P-glycoprotein) by the effect of Piperine (20). The enhancement of systemic exposure and therapeutic effect for the drugs that are co-administrated with Piperine will affect the therapeutic effect of drugs that are co-administrated with Piperine.

The rationale for combining the two herbal medicines P. kurroa and P. longum is to obtain synergistic benefits through the concept of molecular complementarity. The hepatoprotective activities exhibited by picrosides and their related compounds are mediated through antioxidant, anti-inflammatory and hepatoregeneration mechanisms, whereas Piperine acts to supplement the bioavailability of picrosides and to modulate the inflammatory and oxidant pathways associated with liver injury (21). The benefits derived from the combined use of the two herbal medicines could result in a more potent hepatoprotective action

against hepatic injury by virtue of the fact that they target different molecular mechanisms involved in injury and therefore may yield greater efficacy while lowering the dose and toxicity.

3. Hepatotoxicity: Pathophysiology and Molecular Mechanisms

Hepatotoxicity is being caused by any of the following mechanisms: oxidative stress and reactive metabolites created from drug activation; inflammation due to damage to cells or tissues; apoptosis caused by cell death; and fibrosis caused by scar formation. These processes are highly interrelated, and understanding them provides insight into how we might establish multi-target hepatoprotective strategies.

Drug-induced liver injury (DILI)

Drug-induced liver injury (DILI) is an important cause of acute liver failure and the most common reason a drug is withdrawn from the market. DILI occurs in two ways, either as an intrinsic type or as an idiosyncratic (as a result of immune-mediated reactions) type. For many hepatotoxic drugs, the bioactivation of the drug occurs through the action of cytochromes P450 to create reactive metabolites that irreversibly react with cellular macromolecules to cause mitochondrial dysfunction, deplete glutathione, and generate excessive reactive oxygen species. By generating ROS, the liver will further damage itself, thereby increasing the extent of necrosis or apoptosis and increasing the level of inflammatory signals in the in the liver (4).

Alcohol-induced hepatotoxicity

Chronic alcohol consumption induces liver injury through complex metabolic and inflammatory mechanisms. Ethanol metabolism via alcohol dehydrogenase and the microsomal ethanol-oxidizing system generates acetaldehyde and ROS, both of which promote lipid peroxidation and protein adduct formation. Acetaldehyde-mediated modification of cellular proteins impairs mitochondrial function and antioxidant defenses, while gut-derived endotoxins activate Kupffer cells, leading to the release of pro-inflammatory cytokines such as TNF-α (22); (23). These processes contribute to steatosis, steatohepatitis, and progressive fibrosis.

Non-alcoholic fatty liver disease

Obesity, insulin resistance, dyslipidaemia and type 2 diabetes are all potential contributors to the development of NAFLD (non-alcoholic fatty liver disease). NAFLD is a metabolic disorder of the liver that can progress from simple steatosis or fat accumulation to more severe forms of disease like non-alcoholic steatohepatitis (NASH). The progression of NAFLD has been referred to as a multi-hit process because of the involvement of different factors in disease progression, including oxidative stress, lipotoxicity, mitochondrial dysfunction, and chronic inflammatory processes. Free fatty acids (FFAs) added to the liver (hepatocytes) produce ROS from mitochondria and create stress on the endoplasmic reticulum (ER), both of which activate inflammatory and apoptotic pathways as outlined in the following references (24); (25). As a result, liver injury resulting from these "hits" leads to chronic, persistent damage that culminates in fibrinogenic responses (fibrosis) and ultimately cirrhosis.

Viral hepatitis complications

Chronic liver damage occurs primarily due to immune-mediated mechanisms (immune-mediated hepatitis) instead of direct cytopathic virus effects. The continued presence of a virus in the liver leads to the establishment of chronic inflammation through the continued activation of immune cells and the production of pro-inflammatory cytokines. The prolonged inflammatory state causes hepatocellular (hepatocyte) apoptosis and necrosis and stimulates activation of hepatic stellate cells (HSCs), the primary source of fibrogenesis. Therefore, there is an increased risk of developing cirrhosis and hepatocellular carcinoma with this enhanced fibrogenic response (26); (27); (28).

Oxidative stress and lipid per oxidation

Oxidative stress is the main factor attributed to many cases of hepatotoxicity. Oxygen free radicals (ROS), in excess concentrations, will overwhelm the body's own system of antioxidants, such as glutathione and superoxide dismutase, and will cause cellular membranes to lose their integrity by initiating lipid peroxidation of polyunsaturated fatty acids, which can cause further cellular injury via the production of toxic aldehydes, such as malondialdehyde (MDA) and 4-HNE (29); (30).

Inflammatory cascades (NF-κB, cytokines)

Inflammation is highly involved in the progression of liver injury. The activation of several oxidative stress-responsive transcription factors, including NF-κB, promotes the production of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-6. Through the recruitment of immune cells, these factors increase oxidative stress and induce apoptosis of hepatocytes. This continual activation of inflammation, also known as inflammation-mediated liver disease (IMLD), is a significant contributing factor to the pathogenesis of chronic liver disease (31); (32); (33).

Apoptotic pathways

Apoptosis is one of the two primary forms of cell death responsible for the loss of hepatocytes in both acute and chronic liver injury. Hepatotoxic agents' cause the activation of both endogenous (via the mitochondria) and exogenous (via death receptor signaling) forms of apoptosis (34); (35); (36). Induction of apoptosis via the intrinsic pathway results from mitochondrial membrane permeabilization and subsequent release of cytochrome c into the cytosol followed by the activation of caspases. Similarly, extrinsic pathway induction is mediated by the binding of death ligands to their respective death receptors (Fas ligand and Tumour Necrosis Factor). The balance between pro- and anti-apoptotic proteins, such as those of the Bcl-2 family, determines how much hepatocyte apoptosis will occur (37).

Fibrogenic mechanisms

Regardless of the initiating event that leads to chronic liver injury, all marketed isolates of liver fibrosis can be classified as a common pathological consequence of chronic liver injury. Continued damage to the hepatocytes in conjunction with inflammation causes the hepatic stellate cells (HSCs) to change into myofibroblast-like cells that produce large amounts of extracellular matrix components (ECM) such as collagen. Fibrogenic effectors (TGF-beta, PDGF) also play an essential role in activating HSCs and the deposition of ECM and cause pathological changes in the liver architecture, cirrhosis (38); (39).

4. Hepatoprotective Mechanisms of Picrorhiza kurroa

Picrorhiza kurroa's hepatoprotective capability is due to Picrorhiza kurroa modulating several molecular targets associated with liver damage. The bioactive components of Picrorhiza kurroa, most notably iridoid glycosides, provide wide-ranging liver protection by being antioxidants, anti-inflammatories, immunomodulators, and antifibrotics (40).

4.1 Antioxidant Activity

Free radical scavenging mechanisms

Oxidative stress is a major contributor to hepatotoxicity; however, P kurroa has a potent free radical scavenging ability towards reactive nitrogen and oxygen species. The Picrosides and related phenolic components in Picrorhiza kurroa are capable of scavenging superoxide anion radicals, hydroxyl radicals, and peroxynitrite and thus, help limit oxidative insult to lipids, proteins, and DNA in hepatocytes (41); (42).

Enhancement of endogenous antioxidant enzymes

Scientific evidence confirms that endogenic antioxidant systems, containing enzymes such as SOD, CAT, GPx and GSH, have been significantly elevated by P. kurroa. Through the use of these enzymes, lipid peroxidation can be avoided, and redox balance is maintained, as shown in experimental studies utilizing substances or agents known to injure the liver, including drugs or toxins (43); (44).

Nrf2 pathway activation

The major mechanism of action for the proposed antioxidant activity of P.kurroa is the activation of the Nrf2 pathway. Following activation, the Nrf2 protein, which is located in the cytoplasm, will translocate or migrate into the nucleus of the cell, resulting in the transcription or synthesis of protective proteins known as cytoprotective genes, which code for several antioxidant enzymes and Phase II detoxification enzymes. P.kurroa provides additional means of protecting cells from oxidative injury through activations of the Nrf2 pathway (45);(46).

Mitochondrial Protection

Mitochondrial dysfunction provides an environment in which hepatotoxicity may occur. In addition to its many mechanisms protecting against oxidative stress, P.kurroa also protects the mitochondrial membrane from damage by inhibiting the opening of the mitochondrial permeability transition pore and preventing the loss of ATP. The protective effects of P.kurroa will reduce the formation of large quantities of reactive oxygen species (ROS) and therefore reduce or prevent hepatocyte apoptosis (47); (48).

4.2 Anti-inflammatory Effects

NF-κB pathway modulation

P. kurroa blocks NF-κB (the main cause of inflammation) from migrating from the place where it is activated to the nucleus, which in turn, stops it from activating many pro-inflammatory mediators which are involved in damaging the liver (49);(50);(51).

Regulating Cytokine Levels

Treatment with P. kurroa has been shown to reduce TNF-α, IL-6 and IL-1β (pro-inflammatory cytokines) levels significantly. Since TNF-α, IL-6 and IL-1β are involved in regulating immune cell functions, bringing TNF-α, IL-6 and IL-1β back to normal as well as stopping additional pro-inflammatory cytokine release, will help avoid damage to hepatocytes by eliminating damage to the liver caused by immune-mediated mechanisms (52); (53).

Inhibition of COX-2 and iNOS

During liver inflammation, the levels of iNOS and COX-2 are increased and this results in the generation of increased levels of oxidative stress. P. kurroa's active components will block the synthesis of these inflammatory factors, thereby blocking the release of excessive levels of nitric oxide and prostaglandins resulting from iNOS and COX-2 production, which are toxic to hepatocytes (54); (55).

4.3 Membrane Stabilization and Cellular Protection

Prevention of hepatocyte membrane damage

Liver damage is caused primarily by lipid peroxidation (also known as the oxidative breakdown of lipids), which causes destabilisation of the cell membrane of hepatocytes. Therefore, Phyllanthus kurroa (P. kurroa)

protects the hepatocyte cell membrane against oxidative damage and preserves the hepatocyte cell structure and function (56); (57).
P. Kurroa protects liver bile acid homeostasis by promoting bile acid metabolism and secretion. Bile acid homeostasis protects the liver from cholestatic disease and aids the liver in performing its detoxification functions (58).

4.4 Immunomodulatory Properties

Regulation of T-cells and B-cells:
Several chronic liver diseases are due to immune impairment with the most common being viral hepatitis via T cell immunity and autoimmune hepatitis due to an abnormality of T cell immunity and CTLA-4 B cell immunity. P. Kurroa affects T-cell and B-cell proliferation regulation which modulates immunity for hepatocytes' destruction by preventing an excess of hepatocyte damage mediated by T-cells or B-cells (59); (60).

Kupffer Cells are a type of activated Macrophage and are found in the Liver as a resident macrophage that provides a health benefit to the overall body. Kupffer cells, like all macrophages, contribute to liver injury through the release of pro-inflammatory cytokines, reactive oxygen species and the modulation of an immune response from an inflammatory to a protective mode. P. Kurroa modulates macrophage activation by decreasing the release of pro-inflammatory cytokines and reactive oxygen species while also promoting a more protective anti-inflammatory phenotype (61).

Transforming Growth Factor Beta (TGF-β) is a Central Mediator of Fibrogenesis in the Liver. P. kurroa has been shown to inhibit the TGF-β pathway, and thus, inhibit profibrotic gene expression through TGF-β signaling.

Collagen synthesis regulation

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Macrophage activation modulation

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4.5 Anti-fibrotic Activity TGF-β pathway inhibition

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5.1 Bioavailability Enhancement

Piperine’s effect on drug metabolism

Piperine enhances the oral bioavailability of many phytoconstituents and drugs. Piperine increases the concentration of drugs in the plasma, increases their half-life and decreases their metabolism via intestinal absorption and hepatic metabolism (65). This is important when discussing the synergistic effect of piperine in enhancing the efficacy of bioactive compounds from plants with low oral bioavailability as a result of the liver detoxifying capacity before it reaches the systemic circulation.

P-glycoprotein inhibition

Piperine inhibits P-glycoprotein (P-gp), an ATP-dependent efflux transporter expressed in intestinal epithelial cells and hepatocytes. Inhibition of P-gp reduces the efflux of xenobiotics and therapeutic agents back into the intestinal lumen, thereby enhancing intracellular accumulation and systemic exposure. This mechanism contributes significantly to improved bioavailability of hepatoprotective compounds (66).

CYP450 enzyme modulation

Piperine inhibits several CYP450 isoenzymes including CYP3A4, CYP2E1 reducing reactive metabolite formation that leads to hepatic cellular injury. Piperine's modulation of the CYP450 enzymes increases the stability of drugs and reduces oxidative stress resulting from hepatotoxin exposure to hepatocytes (67).

5.2 Antioxidant and Free Radical Scavenging

Direct ROS neutralization

P. longum is known for having strong antioxidant properties by eliminating harmful reactive oxygen species (ROS) including superoxide anions, hydroxyl radicals and hydrogen peroxide. Piperine and other alkaloids act as electron donors to eliminate free radicals, thus reducing oxidative-related damage to components of the liver (68); (69)

Antioxidant enzyme upregulation

P. longum improves the body's natural antioxidant defenses by stimulating the production of superoxide dismutase, catalase and glutathione peroxidase and by enhancing the amount of reduced glutathione available to the cells. The enhanced natural antioxidant system helps to restore the body's redox balance after exposure to chemical agents leading to liver injury (70)

Lipid peroxidation inhibition

Lipid peroxidation is indicative of liver injury; therefore, inhibiting excessive lipid peroxidation will help in preserving membrane integrity and inhibiting hepatocyte injury. Supplementation with P. longum significantly inhibits the formation of malondialdehyde and other products of lipid peroxidation, thus preserving hepatocyte membrane integrity, preventing hepatocyte dysfunction and protecting against liver injury (71); (72).

5.3 Anti-inflammatory Actions

Inflammatory mediator suppression

The release of cytokines and the ongoing activation of immune cells due to inflammation leads to further damage to the liver. The addition of P. longum helps to decrease the levels of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β through the inhibition of NF-κB–signaling pathways. This will help to reduce inflammation in the liver and therefore help to slow down the progression of the damage being caused to the liver (73).

Leukocyte infiltration reduction

Experimental data has shown that P. longum decreases the amount of leukocyte infiltration into the liver by reducing surface adhesion molecules and chemokines. This will decrease the amount of secondary tissue that is damaged by the action of the activated immune cells and will allow for the regression of damaged liver architecture (74); (75).

5.4 Hepatocyte Regeneration

Cell proliferation stimulation

Hepatocyte regeneration is a critical step in the recovery of the liver after it has been injured. Several studies have shown that P. longum decreases the time it takes for the liver to again become normal by increasing the rate of hepatocyte proliferation. The increase in the rate of hepatocyte proliferation is due to the increase in hepatic energy levels and modulation of growth factor-related pathways (76).

DNA and protein synthesis enhancement

P. longum also increases the amount of DNA and protein that is synthesized in hepatocytes, which is attributed to improved nutrient absorption and increased metabolic efficiency. Such increases in the synthesis of DNA and proteins will aid in the repair of damaged hepatocytes and ultimately contribute to the recovery of normal liver function after exposure to toxic insults (77).

5.5 Detoxification Support

Phase I and Phase II enzyme modulation

Coordination between Phase I and Phase II enzyme systems is crucial for detoxifying foreign materials within the liver. Phase I reactions can be improved by the action of P. longum, and the Phase II conjugation pathway can be upregulated to allow the safe removal of xenobiotic by-products (78).

Glutathione metabolism enhancement

Glutathione is an essential determinant of the liver's detoxification ability. By raising the amount of glutathione inside the liver cells, P. longum aids in regenerating glutathione. This improves conjugative reactions and protects liver cells from damage from both oxidative and electrophilic damage (79).These actions of P. longum demonstrate the multiple ways it protects the liver as a metabolite enhancer, metabolic modifier, and hence as a part of combination therapy for the treatment of liver disorders.

6. Preclinical Evidence

There is a considerable amount of in vitro and animal model evidence that support the ability of either Picrorhiza kurroa or P. longum alone, and in combination, to provide hepatoprotective effects. Studies have been conducted using both in vitro analysis of chemical mechanisms and various in vivo animal models mimicking human liver disease processes (80).

6.1 In Vitro Studies

Cell line models

Human hepatocellular carcinoma (HCC) cells, such as the HepG2 cell line, as well as primary rat and human hepatocyte models, have been demonstrated through multiple in vitro investigations to provide useful models for studying the mechanisms of hepatoprotection. P. kurroa extracts and isolated compounds have been shown to provide cytoprotection against chemically induced oxidative damage by restoring hepatocyte viability and protecting their membrane integrity. Additional studies utilizing P. longum have demonstrated

that alkaloids like piperine from that plant have protective effects against the oxidative effects of reactive oxygen species (ROS) on hepatocytes (81).

Mechanism elucidation studies

The molecular mechanisms that mediate hepatoprotection by P. kurroa and piperine have been elucidated using a series of cell-based assays. Reports indicate that P. kurroa compounds decrease intracellular production of ROS and stabilize mitochondrial membrane potential; inhibit NF-κB activation; and modulate and enhance the activities of the transcription factors that regulate antioxidant response element (ARE) genes, such as Nrf2 and heme oxygenase-1. The hepatoprotective effects of piperine have also been attributed to alterations in cytochrome P450 (CYP450) expression and decreases in the bioactivation of certain hepatotoxins within cultured rodent hepatocytes (82).

Dose–response relationships

The in vitro study showing the cytoprotective effects of dosage-dependent increases in the concentration of Picrosides or standardised P. Kurroa extracts demonstrates that higher concentrations lead to greater cell survival and lower levels of lipid peroxidation until reaching a threshold level at which there is no further observed benefit. Similarly, Piperine has exhibited both concentration dependent antioxidant and anti-inflammatory activity, demonstrating the need for optimum dosing when used in the context of combination therapies (83).

6.2 In vivo Animal Models

Carbon tetrachloride (CCl_4)-induced hepatotoxicity

The CCl_4 hepatotoxicity model has been widely used for the study of oxidative stress-induced injury in the liver. In the CCl_4 model system, the use of P. kurroa has produced significant reductions in the elevation of transaminases in serum, as well as in the levels of lipid peroxidation and histological damage. The use of P. kurroa has also been shown to increase the activity of antioxidant enzymes and to suppress the production of inflammatory markers. Similarly, P. longum has been shown to reduce hepatocellular injury and oxidative damage caused by CCl_4 (84).

Paracetamol/acetaminophen-induced liver injury

Paracetamol causes liver toxicity by depleting glutathione levels in the body and creating mitochondrial injury. Preclinical studies have shown that P. kurroa has the capability of raising glutathione levels in the liver, decreasing necrosis and normalizing liver enzyme levels (ALT and AST). In addition, P. kurroa has been found to have the ability to suppress the enzyme CYP2E1, which is responsible for bioactivation of paracetamol, thus decreasing the production of harmful metabolites and increasing survivability (46); (85).

Alcohol-induced liver damage

In animal studies where ethanol was administered, P. kurroa has been shown to reduce oxidative stress produced by ethanol, the release of inflammatory cytokines as a result of the administration of ethanol, and decrease liver fatty changes (steatosis). In addition, supplementation with P. longum decreases fat within the liver, decreases the production of TNF-α, and increases the antioxidant capacity of the liver, all of which have been shown collectively to decrease the progression to alcoholic steatohepatitis (86).

Bile duct ligation models

When bile ducts are tied off, it causes cholestatic liver damage which is characterized by the build-up of bile acids, swelling and scarring of the liver tissue. Animal studies have shown that P. kurroa can help

promote bile flow, lessen the free radical damage caused by cholestatic liver injury, and prevent the formation of scar tissue in the liver. Also, it appears that piperine (extracted from pepper) may also affect the levels of different types of inflammatory mediators in addition to promoting the level of free radical protection during cholestatic injury (87).

NAFLD/NASH models

NAFLD and NASH models have been used to help demonstrate how these herbs can benefit a person's metabolism. For example, in the diet induced NAFLD model of P. kurroa, P. kurroa was able to improve oxidative stress and fatty changes in the liver as well as inhibit several pathways of c-myc induced inflammation, thus slowing disease progression. Meanwhile, P. longum was able to improve the effect of insulin and also promote antioxidant levels relative to control animals (88).

Comparative efficacy studies

A wide variety of comparative studies have shown that P. kurroa demonstrated almost all the same benefits and/or superior benefits compared to standard hepatoprotective agents, such as silymarin, when examined in terms of restoration of biochemical and histological parameters. P. kurroa and P. longum were often used together in these studies, leading to an enhanced protective effect(s), suggesting that the combination of herbs may have either additive or synergistic protective effects (89).

6.3 Synergistic Studies

Combined formulation evaluations

Preclinical research has demonstrated that combining P. kurroa and P. longum is more effective in protecting the liver than using either extract alone, evidenced by a greater ability to normalize liver enzymes, improve antioxidant levels and histological recovery, when studies were conducted on laboratory models of liver damage induced by toxins (90).

Pharmacokinetic interactions

Pharmacokinetic studies have shown that piperine enhances the oral bioavailability of picrosides by preventing their breakdown through metabolism and by effluxing them back into the intestines and liver. By doing so, there is an increase in the amount of picrosides available to the body over time and how long they will last in the body (91).

Enhanced therapeutic outcomes

When combined, P. kurroa and P. longum show a synergistic effect resulting in the following enhanced therapeutic results, such as decrease in oxidative stress, reduction of inflammation, increased regeneration of hepatocytes, and decreased production of scar tissue (fibrosis). Therefore, the rationale for developing multi-targeted herbal formulations is validated through these findings, and should be studied in clinical trials (92).

7. Clinical Evidence and Human Studies

Clinical trials on Picrorhiza kurroa

Evidence for the use of P. kurroa (Kutki) for treating infections has been studied on humans but limited to poor quality data primarily derived from an older clinical setting, including studies and clinical reports that were conducted prior to the introduction of the Controlled Trials Registry (CHARA). Studies conducted on men and women infected with hepatitis via virus, conducted between 2006 and 2023, found improvement in sympto-biochemistry following treatment with Kutki formulations. However, most of these studies do

not conform to current reporting standards used by the CLASS (Clinical Trials and Study Reporting). Many have used small sample sizes and have insufficient sample size and study length, to see the affect of Kutki on patients diagnosed with hepatitis. A recent clinical report comparing Picroliv (a standardized fraction of P. kurroa) for acute viral hepatitis (14 patients studied) indicated symptomatic and biochemical improvement in patients treated with Picroliv (100 mg bid x 4 weeks). A separate clinical report studied the adjunct benefit of routine use of Kutki supplements by patients taking lipid-lowering medications (statins), evaluated for the potential for reducing the adverse effects associated with taking statins based on measuring levels of transaminases and bilirubin. The findings reported in this clinical evaluation provide some evidence for the safety and efficacy of using Kutki supplements for this indication, but cannot be viewed as isolated use of Kutki only (93).

Table 1: Clinical Evidence on Picrorhiza kurroa and Piper longum in Human Studies

Plant / Compound	Study type & population	Formulation / Standardization	Dose & duration	Key outcomes	Major limitations
Picrorhiza kurroa (Kutki)	Clinical study in **acute viral hepatitis** patients	**Picroliv** (standardized iridoid glycoside fraction)	100 mg twice daily, 4 weeks	Improvement in clinical symptoms and reduction in serum bilirubin and transaminases	Small sample size, short duration, older study design, limited mechanistic biomarkers
Picrorhiza kurroa	Clinical evaluation in patients on **long-term statin therapy**	Kutki-based Ayurvedic preparation (polyherbal context)	Variable; formulation-dependent	Protection against statin-associated elevation of liver enzymes	Not a Kutki-only intervention; lack of randomization and blinding
Picrorhiza kurroa	Observational/experience-based reports in hepatic disorders	Crude or semi-standardized extracts	Not uniformly reported	Symptomatic relief in jaundice and chronic liver complaints	Heterogeneous preparations; absence of modern trial endpoints
Piper longum / Piperine	**Human pharmacokinetic study** in healthy volunteers	Piperine-containing herbal formulation	100–200 mg (single/short-term dosing)	Demonstrated systemic exposure and PK feasibility of piperine	Not disease-specific; not focused on liver endpoints
Piperine (adjunct)	Randomized trials in **metabolic/inflammatory conditions** (liver enzymes as secondary outcomes)	Curcuminoids + piperine combinations	Product-specific dosing	Reduction/trends in ALT and AST; improved inflammatory markers	Effects not attributable solely to piperine

Piper longum	Traditional/ethnopharmacological human use	Whole fruit powder or formulations	Variable	Reported digestive and metabolic benefits supporting liver health	Lacks controlled clinical validation

8. Comparative Analysis with Standard Hepatoprotective Agents

A comparative evaluation of Picrorhiza kurroa and Piper longum with established hepatoprotective agents highlights important differences in mechanism, scope of efficacy, safety, and translational applicability.

Silymarin (Milk Thistle)

Silymarin, a flavonolignan complex obtained from the seeds of the milk thistle plant, Silybum marianum, has been one of the most popular herbal products used to protect the liver as it has antioxidant, membrane stabilizing and anti-inflammatory actions and helps prevent liver damage caused by toxins and drugs. Unfortunately, there have been inconsistencies in the clinical outcomes resulting from the use of silymarin due in part to the poor oral bioavailability of the product and the variability in the degree of standardization of the extract used to provide silymarin. In contrast, preclinical studies with P. kurroa have demonstrated equal or greater antioxidant and anti-inflammatory properties compared to silymarin, while P. longum has provided unique benefits to an increased bioavailability of co-administered agents through the component piperine, which is a major limitation with silymarin (94).

N-acetylcysteine (NAC)

N-acetylcysteine (NAC) N-acetylcysteine is the standard agent used in the acute treatment of hepatotoxicity from paracetamol since it is a precursor to glutathione and also functions as a free radical scavenger. While it has been demonstrated that the administration of N-acetylcysteine during the early stages of hepatotoxicity has great efficacy, it has limited utility except for specific indications and little effect on chronic liver disease. Conversely, the administration of P. kurroa both replenishes glutathione levels and simultaneously modulates inflammatory, apoptotic, and fibrogenic pathways, suggesting that P. kurroa may have a more extensive hepatoprotective spectrum than NAC. Further enhancing the hepatoprotective effects of P. kurroa is the ability of P. longum to decrease the bioactivation of toxins by modulating cytochrome P450 enzymes (95);(96).

Ursodeoxycholic acid (UDCA)

The choleretic agent Ursodeoxycholic acid (UDCA) is currently used to treat cholestatic liver diseases. It promotes bile flow from the liver to the intestine, stabilizes liver cell membranes, prevents the cytotoxic effects from excess bile salts, and prevents the cytotoxic effects from certain drugs or chemicals. Although UDCA is effective for the treatment of certain conditions, such as Primary Biliary Cholangitis (PBC), the use of UDCA for many other chronic liver diseases, including Non-Alcoholic Fatty Liver Disease (NAFLD) and advanced liver fibrosis, has limited benefit compared to P. kurroa (including its antioxidant properties and its ability to reduce inflammation). When combined with P. longum, it may provide additional metabolic and bioenhancing effects that are not present with UDCA therapy alone (97).

Synthetic hepatoprotective drugs

Many synthetic drugs available today claim to offer hepatoprotective properties. Most currently-used synthetic agents are effective against one specific mechanism (e.g., oxidative stress and inflammation); they are typically expensive, and produce variable results in the long-term treatment of patients. The majority of

synthetic agents do not exhibit the same broad-spectrum multi-target mechanisms of action exhibited by P. kurroa and P. longum. In addition to targeting oxidative stress, inflammation, and the apoptosis of hepatocytes, these agents can enhance hepatocyte detoxification, inhibit fibrogenesis and promote liver cell regeneration. Thus, they may mimic some of the complex mechanisms that lead to liver disease development. Therefore, while standard hepatoprotective agents are still needed in particular clinical situations, the presented comparative analysis found promising evidence regarding the use of Picrorhiza kurroa and Piper longum as complementary or alternative options that provide broader mechanistic coverage for patients suffering from liver disease. Additionally, there needs to be properly designed clinical studies supporting the efficacy, safety, and optimal utilization of these substances when incorporated into an evidence-based approach for managing liver disease (98).

9. Pharmacokinetics and Bioavailability

Pharmacokineticsof Picrorhiza kurroa constituents and the bioenhancing role of Piper longum will form the basis for the rationale in creating appropriate doses of Picrorhiza kurroa in combination with Piper longum, to predict how these substances behave in other mammals in a clinical context as well as for optimizing treatment regimens for humans.

Absorption, distribution, metabolism, and excretion (ADME)

Iridoid glycosides are moderately polar compounds, with moderate passive intestinal permeability leading to highly variable oral bioavailability. After oral dosing, systemic concentrations are also reduced by gastrointestinal hydrolysis and first-pass metabolism (99). Conversely, the piperidine alkaloids (i.e., piperine) of P. longum are very lipophilic and are rapidly absorbed through the gut (100).

Once absorbed, picrosides preferentially distribute to the liver due to their hepatotropic effect. In animal studies, picroside concentrations in the liver are substantially higher than in plasma, indicating that the hepatic site of action for the pharmacological activity of picrosides is the liver and is responsible for liver detoxification (101). Piperine is also concentrated in the liver; therefore, it may be an important contributor to the regulation of metabolic enzymes and transporters in the liver (102).

Phase I biotransformation of Picrosides typically occurs through hydrolysis and oxidation, followed by conjugation with sulfate (primarily) and glucuronate in Phase II; This rapid biotransformation results in shorter systemic half-lives compared to other compounds. Piperine primarily undergoes hepatic metabolism through cytochrome P450 enzymes CYP2C9 and CYP3A4 to create Hydroxylated and conjugated metabolites (103).

Metabolites of Picroside are generally eliminated from the body via the biliary route, and a smaller amount of elimination occurs through renal excretion; On the other hand, Piperine and all its metabolites are excreted both via biliary and renal systems. The biliary route is efficient in eliminating Piperine because it is found in the hepatobiliary locations and helps with the detoxification process (104).

Piperine’s bioenhancement effects on picrosides

Piperine as a Bioenhancer for Picrosides Piperine is a widely recognized natural bioenhancer that contributes significantly in increasing the bioavailability of Phytochemicals when they are co-administered. Piperine works as a bioenhancer to Picrosides by inhibiting the action of Drug metabolizing enzymes present in our intestines and livers, especially cytochrome P450 3A4 and UDP-Glucuronosyltransferases. This inhibition decreases the amount of drug that is lost due to the first pass metabolism (105). In addition, piperine also inhibits the action of p-glycoprotein which is responsible for the efflux of drugs out of enterocytes and hepatocytes, thus retaining more of the picroside inside the cells. Due to the combined effects of holding picroside in the cells longer and reducing first pass metabolism, the interaction of Piperine

with Picrosides results in a greater peak plasma concentration of these constituents, an increased duration of action systemically and an increased rate of hepatic absorption of these compounds (106).

Tissue distribution patterns

Piperine shows a greater tissue distribution than that of picrosides. Piperine has been shown to be localized within the liver, intestinal tissue and adipose tissue and may assist with the systemic anti-inflammatory and metabolic processes of piperine. Co-administration of piperine has also been shown to increase the hepatic tissue concentrations of phytochemicals that were also simultaneously administered with piperine. This underscores the rationale for combining multiple phytochemicals to produce enhanced therapeutic effects (107).

Metabolic pathways

On a molecular level, picrosides are primarily metabolized through the hydrolytic cleavage of glycosidic bonds, as well as through oxidative metabolism and conjugation processes, which are responsible for the elimination of the substance from the body. All three metabolic pathways are subject to modification through the use of enzyme inhibitors. Picrosides are metabolized through both the hydrolytic cleavage of glycosidic bonds and their oxidative metabolism and conjugation processes. Piperine interferes with the metabolism of picrosides by inhibiting the activity of the principal CYP450 enzymes involved in the oxidative metabolism of picrosides, thus decelerating the rate at which picrosides are eliminated. Although piperine provides therapeutic effects through this deceleration of picroside elimination, it is critical to evaluate the potential for herb-drug interactions in clinical patients who may be receiving multiple medications (103).

10. Safety, Toxicology, and Adverse Effects

Early data from studies on toxicity, as well as human experience and studies on pharmacokinetic interactions provide an initial base for the assessment of the safety of both herbs, but there remain gaps in our knowledge about them that need to be filled by future research.

Also, there are two major categories of toxicological testing to consider when determining the safety of these plants: (1) the testing of the environmental and physical safety of the whole plants and their extracts, and (2) toxicity testing of the active components of the plants.

Toxicology studies conducted in laboratory animals have shown that P. kurroa extracts and standardized fractions of the plant are relatively safe for use, even at doses that are many times greater than therapeutic dosages. Toxicological evaluations of these extracts show that there were no significant changes in bodyweights, the weights of major organs, the results of blood tests, or significant changes in major organs, after multiple doses of the extracts. Human studies have shown that the herbal products have a favourable safety margin (108).

With regard to P. longum, the studies of the toxicity of P. longum fruit extracts and the active ingredient piperine show a high LD_{50} value, which means that both products are relatively safe when used within the therapeutic range (109).

Genotoxicity and mutagenicity assessments

Although there is limited data available evaluating P. kurroa for its potential to produce genotoxic effects, the data that do exist is generally supportive of a low likelihood of genotoxicity. Genotoxicity studies conducted using both in vitro assays and in vivo methodologies (e.g., micronucleus (MNT) tests) did not show any evidence of a mutagenic or clastogenic effect for the therapeutic doses of P. kurroa. For example, when the herb was tested using standard Ames and chromosomal aberration methods, piperine did not

consistently produce evidence of genotoxicity; however, these studies were conducted within the recommended range of safe concentrations (110); (111). Given the absence of data from long-term carcinogenicity studies conducted in humans, additional evaluations will be needed for long-term use of these substances.

In addition to the genotoxicity data on P. kurroa, another concern when using these products is the potential for interaction with medications. Piperine has been shown to inhibit the activity of certain cytochrome P450 (CYP) enzymes (e.g., CYP3A4, CYP2C9 and CYP2E1) and several efflux transporters (e.g., P-glycoprotein). Although inhibition of CYP enzymes contributes to the bioenhancing properties of piperine, it may also increase the concentration of drugs that are metabolized in the liver; thus, unintended consequences (e.g., increased toxicity) may occur if clinicians fail to consider the potential for an interaction (112).

Drug–herb interactions

Currently, there is no strong evidence linking P. kurroa to clinically significant drug interactions; however, caution should be exercised when using products containing piperine with patients receiving narrowly defined drugs; patients taking multiple medications (i.e., polypharmacy) or long-term pharmacotherapy (Stickel and Schuppan, 2020).

Pregnancy and lactation considerations

The safety of both P. kurroa and P. longum when pregnant and/or nursing is poorly documented and therefore, not conclusive. Many references in Ayurvedic texts would discourage use of any Bitter or pungent herbs while pregnant. Piperine has been found to have uterine stimulatory action when administered at higher doses in animals, hence creating much concern about its use during pregnancy.

Consequently, until specifically indicated and under the supervision of qualified healthcare practitioners, women should not take P. kurroa or P. longum and/or their combination during the time of their pregnancy or lactation.

Overall safety perspective

From an overall safety standpoint, Picrorhiza kurroa and Piper longum as a product are generally safe when utilized according to the proposed therapeutic range, most notably for use in the period of a few weeks to several months. This is based on the recommendation of the American Society of Clinical Oncology in a publication that outlines potential interactions between drugs, the limited human data available concerning the safety of long-term administration of these two herbs and the absence of studies on reproductive toxicity. In light of these issues, in addition to the need for standardised formulations and evidence-based dosages, it will be essential through documented toxicological profiling as well as post-marketing surveillance to verify and continually evaluate the safe utilisation of these herbs for hepatoprotective purposes.

11. Formulation Development and Standardization

For successful therapeutic application of both Picrorhiza kurroa and Piper longum, rational formulation design is needed along with development and delivery methods that assure consistent efficacy, safety and compliance by regulatory agencies.

Extract preparation methods

The methods of extract preparation have a great influence on how active constituents are recovered from plants, including both their yield and the biological activity of the extract. Hydroalcoholic extraction is commonly used to extract large quantities of the iridoid glycoside compounds including picroside I,

picroside II, and kutkoside, while reducing the destruction of heat sensitive materials. Depending on the type of extract from P. kurroa, additional defatting steps, using non-polar solvents, may be taken to remove resins and/or other impurities, prior to concentrating on the glycoside fraction (113).

Piper longum is extracted by either ethanolic or hydroalcoholic extraction, these methods are most commonly used to extract the alkaloidal compounds, such as piperine and piperlongumine. Supercritical fluid extraction has been also investigated to produce high-purity piperine, free from or with very little residual solvent. Optimizing the extraction conditions (e.g., solvent polarity, temperature, time of extraction, and particle size) is the key for producing consistent quality product with maximum quantity of bioactive compounds (114).

Novel drug delivery systems

Novel drug delivery systems are increasing in popularity due to limitations related to poor solubility, stability and bioavailability. Nanoformulation of P. kurroa extracts (i.e.: polymeric nanoparticles, solid lipid nanoparticles) can enhance these properties through improved solubility, controlled release and enhanced hepatic localization as evidenced in preclinical studies

Other methods of encapsulating Picrosides and Piperine included liposomes and phytosomes, which serve to prevent degradation in the gut and improve absorption. Co-encapsulation of constituents from both; P. kurroa and P. longum has the potential to enhance the synergistic effects of the two plants by providing a simultaneous delivery mechanism and maximum exposure to targeted tissue. Thus, these new delivery systems could provide a means of increasing the therapeutic benefit of both plant products with less total active compounds.

12. Molecular Docking and In Silico Studies

Recent molecular modeling and simulation studies demonstrate how Picrorhiza kurroa and Piper longum can exert complementary effects through different mechanisms. Specifically, P. kurroa contains phytochemicals that target cellular oxidative stress and inflammation pathways (i.e., Nrf2 and NF-kB) whereas P. longum (Piper) contains phytochemicals that target drugs and transporters in order to improve bioavailability. In addition, these in silico studies provide evidence supporting the intended use of the combination of both herbs and a rational approach to product development for the establishment of effective, reliable, and, ultimately, safe hepatoprotective therapies based on these two medicinal plants (115).

13. Regulatory Perspectives and Market Status

In order for P. kurroa and P. longum to successfully integrate into standard healthcare practices, there are numerous regulatory and quality assurance barriers that must be overcome. In India, P. kurroa is regulated as an Ayurvedic medicine, as it is supported by traditional use; however, in Western countries, P. longum is primarily classified as a dietary supplement and, therefore, no therapeutic claims are allowed. Many companies are currently marketing both herbs as "liver support" or "bio-enhancing." However, the regulatory landscape regarding the standardization of products and quality control practices is not uniform across countries thus limiting the general acceptance and confidence among healthcare providers regarding the efficacy of these products. Therefore, standardizing both products and establishing a framework for aligning regulatory practices across countries will enhance clinical provider's confidence in using these products and increase the chances that they will be adopted in mainstream healthcare (116);(117).

14. Future Research Directions

Hepatoprotective abilities of Picrorhiza kurroa and Piper longum not only have a historical use in traditional medicine but also lack the supporting development and validation as functional medicinal products through

clinical trials and molecular characterization. The future of these herbal products as evidence-based medicine lies in conducting discovery research of new bioactives using metabolomics technology and fully elucidating their mode of actions with multi-omics methodologies. To facilitate these efforts, investigators must conduct very methodologically sound multicentric randomized trial designs, and assess how best to use these products in personalized medicine. Integration of clinical and preclinical research will create a much stronger case for approval of these therapies, as will the use of technology-based solutions (e.g., nanomedicine) to improve their bioavailability in vivo.

15. Conclusions

Collectively, a significant body of evidence demonstrates the synergistic protective capabilities of Picrorhiza kurroa and Piper longum toward the liver. P kurroa reduces oxidative stress and inflammation while P longum increases the therapeutic action via bioenhancement of P kurroa. Although there is a strong preclinical/potentially clinical basis for the application of these botanical products in the management of liver disease (particularly in NAFLD and DILI), their widespread adoption remains limited by the scarcity of high-quality randomized clinical studies and the lack of long-term safety studies. Clinical validation (based on high-quality randomized clinical studies) combined with omics technology and additional advanced delivery systems will be vital to substantiating the clinical use of Picrorhiza kurroa and Piper longum for the treatment of liver diseases.

Abbreviations

Abbreviation	Full Term
ADME	Absorption, Distribution, Metabolism, and Excretion
ALT	Alanine Aminotransferase
ARE	Antioxidant Response Element
AST	Aspartate Aminotransferase
CAT	Catalase
CCl_4	Carbon Tetrachloride
COX-2	Cyclooxygenase-2
CYP450	Cytochrome P450
DILI	Drug-Induced Liver Injury
ECM	Extracellular Matrix
GPx	Glutathione Peroxidase
GSH	Reduced Glutathione
HCC	Hepatocellular Carcinoma
HSCs	Hepatic Stellate Cells
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
iNOS	Inducible Nitric Oxide Synthase
LD50	Lethal Dose, 50%
NAC	N-acetylcysteine
NAFLD	Non-Alcoholic Fatty Liver Disease
NASH	Non-Alcoholic Steatohepatitis
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
Nrf2	Nuclear Factor Erythroid 2–Related Factor 2
P-gp	P-glycoprotein
PK	Pharmacokinetics
ROS	Reactive Oxygen Species
SOD	Superoxide Dismutase

TGF-β	Transforming Growth Factor-beta
TNF-α	Tumor Necrosis Factor-alpha
UDCA	Ursodeoxycholic Acid

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