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Review Article

Milk Thistle and its Therapeutic Potential in Liver Disorders: A Comprehensive Review

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Abstract

Liver disorders need effective treatments to enhance function, reduce inflammation, and prevent viral disorders. Silymarin shows promise, but its optimal dosage and duration remain unclear, complicating standardized clinical use. Milk thistle [*Silybum marianum* (L.) Gaertn.] and its active component, Silymarin, has shown its effectiveness in enhancing liver function, mitigating inflammation, and preventing further damage in conditions from alcoholic fatty liver, non-alcoholic fatty, drug-induced liver injury and viral hepatitis. Clinical trials have furnished evidence of silymarin's beneficial effects on liver health, particularly in conditions like cirrhosis. However, it's crucial to acknowledge that while silymarin appears to possess hepatoprotective properties, its optimal dosage and duration of use may vary depending on the specific liver condition. Understanding the therapeutic role of silymarin within the intricate landscape of liver illnesses can contribute to more effective strategies for patient care and improved outcomes.

Keywords: Milk thistle; *Silybum Marianum*; Liver disorders; Clinical trials; Oxidative stress

Introduction

Silybum marianum (L.) Gaertn., commonly known as milk thistle and its derivatives have been employed in the treatment of liver diseases. Reports indicate that up to one-third of individuals use milk thistle compounds as supplementary therapy for chronic viral hepatitis, and clinical observations suggest an even higher prevalence among those with chronic liver disease (Heleen 2022). The historical use of milk thistle seed extracts underscores their longstanding reputation for liver protection (Hackett *et al.* 2013).

Liver diseases are a major global health issue that impacts millions of people and puts an increasing strain on healthcare systems. These diseases are becoming more common, mostly because of sedentary lifestyles, altered eating patterns, and rising rates of risk factors like alcoholism and obesity (Seeff *et al.* 2015). Liver diseases are a group of disorders that include alcoholic fatty liver disease (AFLD), drug-induced liver damage (DILI), viral hepatitis, and non-alcoholic fatty liver disease (NAFLD). Liver cirrhosis is one of the worst consequences; it is an irreversible, chronic disorder marked by significant scarring that affects the essential liver processes (Gu *et al.* 2015).

The death rate related to liver illnesses is rising along with their frequency. Liver-related mortality has increased significantly, especially in midlife, and now accounts for a large number of deaths in many countries. As a result of several liver conditions, liver cirrhosis is one of the world's major causes of morbidity and death. The complex network of liver disorders includes viral hepatitis (Thrift *et al.* 2017), an infectious liver condition with long-term consequences, oxidative liver injury caused by an excess of reactive oxygen species, and non-alcoholic fatty liver disease (NAFLD), characterized by fat accumulation in the liver (Petta *et al.* 2009; Diaz *et al.* 2023). Furthermore, DILI is dangerous, especially when treating diseases like tuberculosis (Table 1) (Goldberg *et al.* 2015).

Humanity has historically looked on both pharmacological and natural therapies (Langmead and Rampton 2001) to treat liver disorders. Milk thistle, renowned for its hepatoprotective qualities, is one such age-old medicine that has endured (Hashem *et al.* 2021). People

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Table 1: Summarizing the mode of action of the primary bioactive compounds in milk thistle focusing on their therapeutic effects on the liver and mechanisms

Compound	Therapeutic Effect	Mechanism of Action	References
Silybin		• Scavenges free radicals, reducing oxidative stress	(Nasiri-Ansari and Kassi 2023)
		• Stabilizes hepatocyte membranes, preventing toxin penetration	
		• Promotes liver cell regeneration by stimulating protein synthesis	
	Anti-inflammatory	• Inhibits NF-κB, reducing pro-inflammatory cytokines and enzymes	(Alvari *et al.* 2012)
	Antifibrotic	• Inhibits hepatic stellate cell transformation, reducing collagen deposition	(Salvoza *et al.* 2022)
Silydianin	Hepatoprotective	• Similar antioxidant and membrane-stabilizing effects as silybin	(Nasiri-Ansari and Kassi 2023)
	Antioxidant	• Enhances endogenous antioxidant levels like glutathione	(Riaz *et al.* 2023)
Silychristin	Hepatoprotective	• Prevents lipid peroxidation, protecting liver cell membranes	(Salvoza *et al.* 2022)
		• Scavenges reactive oxygen species	
Isosilybin	Hepatoprotective	• Similar hepatoprotective mechanisms as silybin	(Salvoza *et al.* 2022)
		• Promotes cell regeneration in liver tissues	
Silymarin Complex	Antioxidant	• Enhances overall antioxidant defense, reducing oxidative damage	(Riaz *et al.* 2023)
	Antifibrotic	• Inhibits stellate cell activation, reducing liver fibrosis	(Salvoza *et al.* 2022)
		• Reduces inflammation by downregulating NF-κB and related cytokines	(Alvari *et al.* 2012)

have been using milk thistle for its possible benefits without knowing its precise components since before science had a clear understanding of the plant. After significant scientific progress, important chemicals were eventually isolated. One of the most well-known is silymarin, extracted from milk thistle seeds (Luper 1998; Adetuyi *et al.* 2021).

Silymarin, an intricate blend of polyphenolic compounds, has drawn notice for its diverse impacts on hepatic function (Saller *et al.* 2007). Different investigations on its characteristics have been conducted over time, including how it reduces inflammation, inhibits the effects of reactive oxygen species, restricts liver fibrosis, and demonstrates antioxidant qualities (Iranikhah *et al.* 2017). Silymarin, the antioxidant component, and Silybum marianum extracts prevent liver damage (Shaker *et al.* 2010). This introduction seeks to present a thorough review of liver illnesses, the historical background of milk thistle use, the identification of silymarin, and the substantial studies carried out to evaluate its effects on various liver ailments. We can enhance patient outcomes and develop more effective strategies by comprehending the complex interactions between liver disorders and the possible therapeutic role of silymarin. The objective of the review is to analyze the prevalence and impact of liver diseases, such as AFLD, DILI, viral hepatitis and NAFLD. Examine the severe consequences of these diseases, including liver cirrhosis and rising liver-related mortality rates. Explore the historical use of milk thistle in liver disease treatment, emphasizing its longstanding reputation for liver protection. Identify and discuss silymarin, the active compound in milk thistle, and its hepatoprotective properties. Summarize findings from clinical studies on silymarin's efficacy in enhancing liver function, reducing inflammation, and preventing liver damage. Discuss the potential therapeutic role of silymarin in managing various liver disorders. Highlight the need for further research to determine optimal dosages and treatment protocols for different liver conditions.

A comprehensive review of the literature was conducted to gather information on the therapeutic significance of *Silybum marianum* (milk thistle) and its derivative silymarin in the treatment of liver diseases, including alcoholic fatty liver disease (AFLD), drug-induced liver damage (DILI), non-alcoholic fatty liver disease (NAFLD), cirrhosis, and viral hepatitis C. Electronic databases including PubMed, Scopus, and Google Scholar (published data), Textbooks, clinical guidelines, and review articles were also consulted to ensure a comprehensive understanding of the subject were systematically searched using relevant keywords such as *Silybum marianum*, milk thistle, silymarin, liver diseases, cirrhosis, alcoholic fatty liver disease, non-alcoholic fatty liver disease, drug-induced liver damage, and hepatitis C. Additional sources such as textbooks, clinical guidelines, and review articles were also consulted to gather comprehensive.

Therapeutic significance of milk thistle

S. marianum is one of the most significant therapeutic herbs globally. Throughout Europe, milk thistle has been used as medicine since the first century. Its fruits (achenes) have long been utilized as a coffee substitute, while its petals, roots, and leaves have all been incorporated into European cuisines as vegetables. For almost 2,000 years, people have used *S. marianum* seeds as a natural treatment for liver and bile duct issues. The pharmacologically beneficial medication made of silymarin consists of four main components: silybin between 50 and 60 percent, silychristin 20 percent, iso silybin 5 percent, and silydianin 10% (Samee *et al.* 2023).

Milk thistle seeds are rich in saturated fatty acids. The seeds may be utilized to provide people with protein and edible oil. However, before suggesting the use of this oil for food, it might require thorough processing and refining (Khan *et al.* 2007).

Chemistry and bioactive compounds of milk thistle

S. marianum produces seeds rich in silymarin, a complex of flavonolignans and other polyphenolic compounds. The primary flavonolignans in silymarin include silybin,

isosilybin (A and B), silydianin and silychristin. Silybin, the most abundant component, has a molecular formula of $C_{25}H_{22}O_{10}$ and a molecular weight of 482.441 (Da) (Bijak 2017). Silybin is a highly functionalized molecule with alternating carbocycles and heterocycles, making it resistant to reduction but easily oxidized to 2, 3-dehydrosilybin. It is stable under acidic conditions but unstable in the presence of Lewis acids or basic conditions. In neutral aqueous solutions, silybin behaves as a weak acid. It contains five hydroxyl groups, which can form esters, ethers, and ketones (in its enol form). The biosynthesis pathway of silybin is not fully understood. Biomimetic reactions suggest that silybin can be synthesized from coniferyl alcohol and taxifolin via peroxidase. Concentration profiles of silybin and its precursors, along with RNA-sequencing analysis, indicate that the amount of taxifolin and peroxidase activity is limiting factors in silybin biosynthesis. Among five peroxidase candidates, Ascorbate Peroxidase 1 showed significant activity in synthesizing silybin (Lv *et al.* 2017). Silymarin also contains a mixture of undefined polyphenolic compounds, often referred to as the "polymeric fraction". Additionally, the oil fraction, which includes linoleic, oleic and palmitic acids, sterols, tocopherol (vitamin E) and phospholipids, has not been fully explored (Chambers *et al.* 2017).

Mechanisms of action of milk thistle in liver disorders

Silymarin, the active component in milk thistle, acts as a potent antioxidant, shielding the liver from harmful free radicals that can damage liver cells. Free radicals are unstable molecules that can target proteins, lipids, and other cellular components, causing cellular damage or destruction. Additionally, silymarin reduces inflammation within the liver by suppressing pro-inflammatory cytokines, which supports a healthier liver environment and prevents chronic inflammation from harming liver tissue over time as mentioned in Table 1 (Abenavoli *et al.* 2018). Milk thistle aids in liver cell repair and regeneration by encouraging the growth of new, healthier liver cells. This regenerative ability is crucial for healing liver damage caused by toxins, medications, or diseases. Furthermore, silymarin strengthens the outer membranes of liver cells, preventing harmful toxins from penetrating the liver and neutralizing liver cell damage caused by toxins, surpassing the antioxidant potency of Vitamin E. Milk thistle also contributes to protein synthesis, which is essential for maintaining liver function and ensuring the liver's ability to perform metabolic processes efficiently. Additionally, it helps reduce fibrotic activity, which occurs when excessive scar tissue forms in the liver due to chronic damage, thus preserving liver function (Ball and Kowdley 2005).

Effect of silymarin in the activity of liver enhancement

Cirrhosis (chronic liver disease): Chronic liver disease cirrhosis is caused by the replacement of healthy liver tissue with scar tissue, which compromises the liver's ability to function. Various conditions, including autoimmune diseases, metabolic abnormalities, viral hepatitis, and alcohol addiction, can lead to cirrhosis. Complications from cirrhosis may include bleeding varices, ascites, hepatic encephalopathy, portal hypertension and liver cancer (Table 2). According to the British Liver Trust, liver disease was the leading cause of death in midlife (ages 45 to 64), accounting for half of all deaths in England in 2020. Individuals aged 65 to 84 accounted for 35% of all deaths, while those aged 25 to 44 accounted for 11%, and those over 85 for 4% of all deaths. Less than 1% of deaths involved individuals younger than 25 (Asrani *et al.* 2019).

Effect of silymarin on cirrhotic liver disease: The antioxidant properties of silymarin help protect liver cells from inflammation and oxidative damage (Saller *et al.* 2007). Additionally, silymarin may modulate the activity of enzymes associated with cirrhosis and liver fibrosis as shown in Fig. 1–3 (Trappoliere *et al.* 2009; Gharagozloo *et al.* 2010; Li *et al.* 2012).

Various clinical trials have demonstrated that silymarin improves liver function (Pelt *et al.* 2003) and reduces liver-related mortality in patients with cirrhosis, particularly those with alcoholic or non-alcoholic fatty liver disease. Silymarin acts as a scavenger of free radicals and modulates enzymes associated with the development of cirrhosis, fibrosis, and cellular damage (Gillessen and Schmidt 2020). Clinical trials involving individuals with alcoholic or non-alcoholic fatty liver disease, including those with cirrhosis, have shown these hepatoprotective effects. A significant reduction in liver-related mortality was associated with silymarin treatment in a pooled analysis of trials involving patients with cirrhosis (Pellicoro *et al.* 2014; Gillessen and Schmidt 2020). Hepatic stellate cell (HSC) activation is a hallmark of hepatic fibrogenesis, resulting from persistent liver tissue injury. In animal models, silybin has been shown to possess anti-fibrogenic properties (Trappoliere *et al.* 2009).

Clinical trials showing the effect of silymarin on cirrhosis

The effects of silymarin on individuals with NAFLD or cirrhosis at the advanced stage of liver fibrosis have been assessed in several clinical trials. Silymarin significantly reduced the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), markers of liver impairment, in individuals with non-alcoholic fatty liver disease (NAFLD), according to a meta-analysis of six trials (Ferenci 2016). Silymarin also improved liver function tests, histological indicators and quality of life in cirrhosis patients, according to another meta-analysis of nine trials. Furthermore, silymarin was shown to reduce insulin resistance and enhance glycemic control in patients with alcoholic cirrhosis and diabetes (Abenavoli *et al.* 2010).

Table 2: Research evidence on the clinical use of milk thistle extracts for treating various liver diseases

Etiology	Liver disease stage	Reference
Viral hepatitis	Acute, chronic, liver failure and cirrhosis	
Alcoholic liver disease	Acute, chronic, liver failure and cirrhosis	(Bijak 2017)
Nonalcoholic liver disease	Acute, chronic, and cirrhosis	
Cholestasis	Pregnancy and nonpregnancy-related	(Abenavoli *et al.* 2018)
Drug- and toxin-induced liver disease	Acute, chronic, liver failure, and cirrhosis	(Gillessen *et al.* 2020)
Primary liver malignancy	Hepatocellular carcinoma and cholangiocarcinoma	

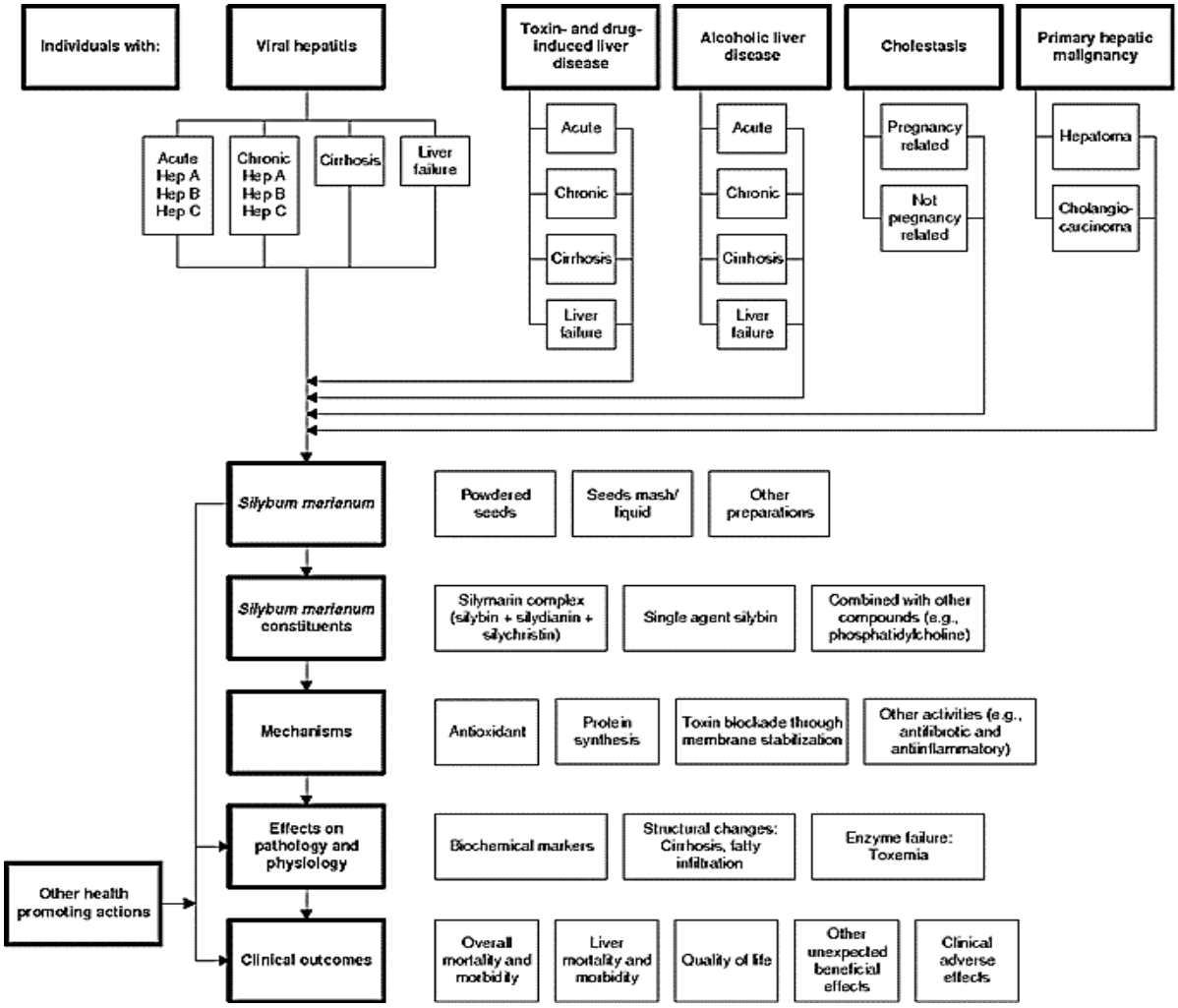

Fig. 1: This diagram illustrates the comprehensive effects of Milk Thistle *(Silybum marianum)* on liver disease, detailing its mechanisms of action, benefits and therapeutic outcomes

The optimal dosage of silymarin for nonalcoholic fatty liver disease (NAFLD) or cirrhosis varies based on the type, stage, and severity of the illness. Throughout most of the clinical trials, dosages ranging from 140 to 420 mg daily for six to 24 months were administered using Eurosil 85®, a unique formulation of silymarin with good oral bioavailability. However, some research suggests that longer periods (up to 48 months) or higher doses (up to 700 mg daily) may be necessary for optimal results. Therefore, determining the ideal silymarin dosage for NAFLD or cirrhosis should be based on the patient's condition and response to therapy (Pradhan and Girish 2006).

The published data assessed the effect of silymarin on the prognosis of patients with cirrhosis through a double-blind, prospective, randomized trial design. With a median follow-up of 41 months, the trial included 170 patients with cirrhosis. Of these, 87 received silymarin 420 mg/day (alcoholic: 47, non-alcoholic: 40) and 83 received a placebo (alcoholic: 45, non-alcoholic: 38) for at least 24 months (Gillessen and Schmidt 2020).

In the silymarin group, liver illness was the cause of 16 out of 28 deaths, while in the placebo group, liver-related

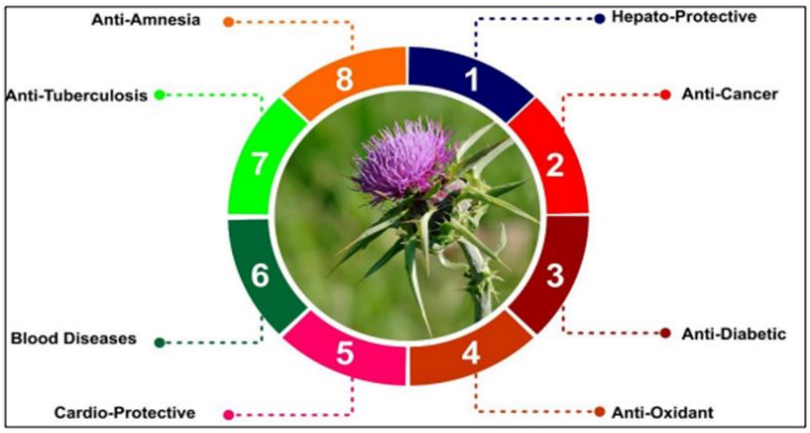

Fig.2: Succinctly illustrates the diverse therapeutic effects of *Silybum marianum* (milk thistle) on different systems in the human body

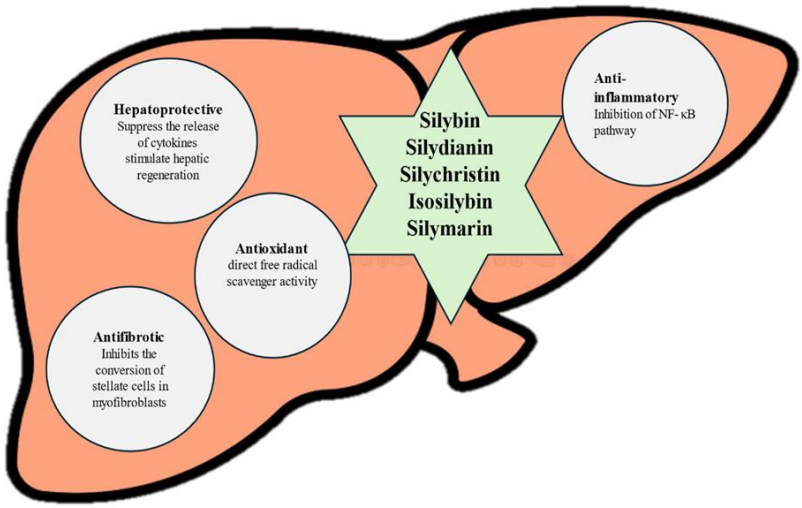

Fig. 3: Therapeutic effects of active compounds from milk thistle on the liver health

diseases accounted for 32 out of 39 deaths. According to the study, patients received silymarin had a significantly higher 4-year survival rate (58% *vs.* 39%) than those who received a placebo (*P = 0.036*). Subsequent analyses revealed that therapy decreased mortality in patients with less advanced cirrhosis (class A based on the Child-Turcotte criteria; *P = 0.03*) as well as in patients with alcoholic cirrhosis (*P = 0.01*) (Child and Turcotte 1964).

Non-alcoholic fatty liver disease

A disorder known as non-alcoholic fatty liver disease (NAFLD) is characterized by the accumulation of fat in the liver cells of individuals who do not consume excessive amounts of alcohol (Younossi *et al.* 2016). It is typically associated with obesity, insulin resistance, type 2 diabetes, elevated blood pressure and elevated cholesterol levels. NAFLD can progress from basic steatosis (fat buildup) to non-alcoholic steatohepatitis (NASH), which involves inflammation and destruction of liver cells. NASH can further lead to cirrhosis, resulting in permanent loss of liver function and fibrosis, characterized by scarring (Chalasani *et al.* 2018).

The exact mechanism by which NAFLD causes liver damage remains unclear, but oxidative stress plays a significant role (Ore and Akinloye 2019). Other contributing factors include mitochondrial dysfunction, inflammation, apoptosis (cell death) and altered lipid metabolism (Wong and Ahmed 2014; Chen *et al.* 2017). These factors may trigger a cascade of events leading to cirrhosis, fibrosis, and liver damage (Hossain *et al.* 2016).

Effect of silymarin in NAFLD and clinical trials

Silymarin benefits patients with non-alcoholic fatty liver disease (NAFLD) by reducing liver fat accumulation (Yao *et al.* 2013), strengthening antioxidant defenses, lowering blood sugar and cholesterol, promoting insulin sensitivity,

reducing fibrosis, and delaying liver cell death (Abenavoli *et al.* 2011). Since the commercial version of silymarin (Eurosil 85®) at the recommended dosage of 420 mg/day did not show significant improvement in pilot testing, a clinical investigation was conducted with a larger dosage of silymarin to treat patients with NASH (Kim *et al.* 2012). Patients with biopsy-confirmed NASH received a 48-week course of treatment with a higher dosage of silymarin (2100 mg/day), which was deemed safe based on earlier phase I research. The trial was randomized, double-blind, and placebo-controlled (Kheong *et al.* 2017). The silymarin and placebo groups did not significantly differ in terms of the primary endpoint, which was a ≥ 30% improvement from baseline in the NASH and NAFLD Activity Score (NAS) on liver biopsy; however, the silymarin group showed a significant improvement in fibrosis. Additionally, a greater number of patients in the silymarin group experienced either resolution or improvement of their fibrosis and there was a favorable change in liver stiffness (change in hepatic stiffness −0.7 *vs.* 6.0 kPa) in the silymarin group; however, these differences were not statistically significant. Noninvasive fibrosis markers (AST to platelet ratio index, fibrosis-4 score, and NAFLD fibrosis score) similarly improved in the silymarin-treated group but not in the placebo-treated group (Sorrentino *et al.* 2015).

Liver oxidative injury

When the liver is exposed to high concentrations of reactive oxygen species (ROS), which can damage cellular components and compromise liver function, the condition known as liver oxidative injury develops (Li *et al.* 2015; Ore and Akinloye 2019). Numerous factors, including alcohol intake, viral hepatitis, non-alcoholic steatohepatitis (NASH), medications, toxins, ischemia-reperfusion and inflammation, can contribute to liver oxidative injury. Hepatocellular carcinoma, cirrhosis, and liver fibrosis, all of which are significant global causes of morbidity and mortality, can result from liver oxidative stress.

According to the World Health Organization (WHO), liver cirrhosis was responsible for around 771,000 deaths in 2001, making it the 14th most common cause of death worldwide. Liver cirrhosis was predicted to rank as the 12th most common cause of death in 2020. In the US, cirrhosis or chronic liver disease accounted for 17 fatalities per 100,000 people in 2019. Timely diagnosis and appropriate therapy are essential for addressing the significant health issue of liver oxidative damage to halt its progression and mitigate its consequences (Noel-Hudson *et al.* 1989).

Effect of silymarin on liver oxidative injury and clinical trials

A hepatoprotective substance called silymarin is extracted from milk thistle (*Silybum marianum*) seeds. It has been utilized for millennia to treat various liver conditions, including cirrhosis, drug-induced liver damage, non-alcoholic fatty liver disease, and alcoholic liver disease. One of its primary modes of action is its ability to scavenge free radicals and reduce oxidative stress in liver cells. Oxidative stress, due to its capacity to promote lipid peroxidation, inflammation, fibrosis, and apoptosis, is a major contributor to liver damage. Silymarin can modulate the activity of several oxidative stress-related enzymes, including glutathione reductase, catalase, superoxide dismutase, and glutathione peroxidase. Additionally, silymarin can elevate the liver's levels of glutathione, an essential antioxidant molecule, thereby reducing oxidative stress, protecting the liver from further damage, and enhancing its regenerative capacity (Surai 2015).

Ninety individuals with hypoxia were admitted to the hospital and studied. The patients ranged in age from 15 to 89, with a mean age of 56.31 ± 20.01 years. Sixty-six percent of the patients were male, while the remaining thirty-four percent were female. There were no statistically significant differences between the age and gender distributions of the silymarin group and the placebo group ($P = 0.656$ for age and $P = 0.5$ for gender), indicating their similarity (Mayer *et al*. 2005).

By comparing the mean and standard deviation of these data on the first and third treatment days in the silymarin and placebo groups, the impact of silymarin on WBC count and GGT level was evaluated. The findings showed that silymarin did not affect the WBC count since there was no significant difference in WBC count between the two groups on any day ($P > 0.05$). However, on the third day, there was a significant difference ($P = 0.0001$) in the GGT levels between the two groups; the silymarin group had lower GGT levels than the placebo group, indicating that silymarin had a beneficial effect on GGT. Moreover, a notable reduction in liver enzymes and GGT levels was observed in the silymarin group between the first and third days ($P < 0.05$), suggesting the advantageous impact of silymarin on liver function (Jiang *et al.* 2021).

Anti-tuberculosis drug-induced liver injury

Rare but potentially dangerous, drug-induced liver injury (DILI) can occur as a side effect of various anti-tuberculosis (TB) medications, leading to jaundice, liver damage and in rare cases, liver failure. Although the exact cause of DILI is unknown, it is believed to be linked to the liver's production of toxic metabolites during the breakdown of anti-TB medications. These compounds can trigger an immunological response that damages liver cells. Factors such as the type and dosage of anti-TB medications, duration of treatment, patient's genetic makeup, presence of other liver conditions, and alcohol consumption can all influence the risk of DILI (Gu *et al.* 2015).

The three most common anti-TB medications associated with DILI are pyrazinamide, isoniazid, and rifampicin. Typically, these medications are used in

combination with ethambutol for the first two months of TB treatment, followed by rifampicin and isoniazid for an additional four months. The incidence of DILI from these medications varies depending on the population and terminology used but is generally estimated to be between 14 and 19 per 100,000 patients receiving anti-TB medication. However, certain medications pose a higher risk than others. For example, the incidences of amoxicillin-clavulanate (43 per 100,000), nitrofurantoin (73 per 100,000), infliximab (675 per 100,000), and azathioprine (752 per 100,000) are higher (Zhong *et al.* 2021).

DILI can manifest in various ways depending on the type and extent of liver damage. The predominant pattern is hepatocellular damage, primarily affecting liver cells and resulting in elevated levels of bilirubin and alanine aminotransferase (ALT). Another pattern is cholestatic damage, mainly affecting bile ducts and resulting in elevated levels of bilirubin and alkaline phosphatase (ALP) (Jiang *et al.* 2021). Additionally, a mixed pattern involving both cholestatic and hepatocellular damage may occur. Diagnosis of DILI relies on clinical criteria such as exclusion of other causes of liver injury, abnormal liver tests above thresholds, improvement or resolution of symptoms and liver tests after discontinuation of the medications, and a history of exposure to anti-TB treatments (Tostmann *et al.* 2008).

Effect of silymarin on DILI and clinical trials

Due to its hepatoprotective, anti-inflammatory, and antioxidant properties, silymarin may protect the liver from the adverse effects of anti-tuberculosis (anti-TB) medications such as pyrazinamide, isoniazid and rifampicin. These medications are commonly used to treat tuberculosis, a chronic infectious disease caused by *Mycobacterium tuberculosis*. However, they can lead to drug-induced liver injury (DILI) in certain patients, resulting in jaundice, liver failure and hepatitis. DILI is one of the most dangerous side effects and a significant reason for the failure of tuberculosis treatment (Luangchosiri *et al.* 2015).

The exact mechanism underlying silymarin's protective action against anti-TB drug-induced liver injury remains unknown, but several pathways may be involved. Silymarin may inhibit or reduce the formation of reactive metabolites of anti-TB medications, which can damage liver cells and cause oxidative stress. Additionally, silymarin may enhance the activity of antioxidant enzymes that scavenge free radicals and protect the liver from oxidative damage, such as glutathione peroxidase and superoxide dismutase. It may also regulate the expression of inflammatory cytokines that could induce inflammation and apoptosis in the liver, such as interleukin-6 and tumor necrosis factor-alpha. Silymarin might also prevent the activation of nuclear factor-kappa B, a transcription factor that regulates the expression of genes associated with fibrosis, apoptosis, and inflammation. Ultimately, silymarin may improve liver function and support liver cell regeneration (Shi *et al.* 2020).

The effectiveness and safety of silymarin as a supplementary treatment for tuberculosis patients receiving anti-TB medications have been evaluated in multiple clinical trials. However, the outcomes are inconsistent and inconclusive. Some studies suggest that silymarin can improve liver function tests, shorten hospital stays and reduce the frequency and severity of DILI (Kren and Walterova 2005).

In a study involving 4304 patients observed over 6–9 months, 2752 patients (63.9%) used hepatoprotection as a prophylactic measure for a median of 183 days. The most commonly used primarily for liver health medications were inosine, glucurone, silymarin, and Hu Gan Pian (Traditional Chinese medicine). The incidence of anti-tuberculosis drug-induced liver injury (ATLI) was 2.5% (37 patients) in the reference group and 2.4% (69 patients) in the preventative usage group. However, the use of hepatoprotection, including silymarin, did not significantly correlate with the incidence of ATLI (Wu *et al.* 2015).

Regarding the incidence of DILI, liver enzyme levels, and treatment outcomes, silymarin and placebo groups did not significantly differ from each other in other studies. The inconsistent findings may be attributed to the heterogeneity of these trials concerning study design, sample size, dosage, duration of silymarin administration, criteria for diagnosing DILI, and outcome assessments. Therefore, more thorough, and carefully planned randomized controlled trials are needed to validate silymarin's therapeutic potential for liver impairment caused by anti-TB drugs.

Viral hepatitis C

The hepatitis C virus, transmitted through exposure to infected blood, causes viral hepatitis C, a liver infection (Alter 2007). The infection can vary in severity from mild to severe and may manifest as either acute or chronic. According to the World Health Organization (WHO), an estimated 1.5 million new cases of hepatitis C infections occur annually, with approximately 58 million individuals worldwide suffering from chronic hepatitis C infection. Serious complications of chronic hepatitis C include cirrhosis, liver cancer and liver failure. In 2019, about 290,000 deaths were attributed to causes related to hepatitis C. However, direct-acting antiviral medications (DAAs) have demonstrated a cure rate exceeding 95% for the infection (Feld and Hoofnagle 2005).

Effect of silymarin on hepatitis c and clinical trails

Patients with hepatitis C may potentially benefit from silymarin's demonstrated anti-inflammatory, antioxidant, antiviral, and immunomodulatory properties (Freedman *et al.* 2011). Silymarin may contribute to increased longevity, improved liver function, and reduced viral load in hepatitis C patients. However, the optimal dosage, duration and

formulation of silymarin have not yet been established, and the evidence remains inconclusive. Moreover, silymarin may have adverse effects such as nausea, diarrhea and allergic reactions, in addition to potential interactions with several antiviral medications used in hepatitis C treatment, such as interferon and ribavirin (El-Kamary *et al.* 2009).

Through comprehensive investigation into silymarin's hepatoprotective qualities, scientists have been able to elucidate a wide range of intricate molecular pathways that regulate its effects on liver health (Wagoner *et al.* 2010). Silymarin, a compound with multiple activities including antiviral, anti-inflammatory, immunomodulatory, and antioxidant properties, is derived from the milk thistle plant. The objective of this research is to delineate the complex effects of silymarin on the hepatitis C virus (HCV) and to provide insights into how it inhibits the virus at various stages of its life cycle (Koltai and Fliegel 2022).

Significant discoveries include the effectiveness of silymarin in blocking HCV entry, fusion, RNA and protein synthesis and the generation of infectious virus particles (Ahmed-Belkacem *et al.* 2010). Silymarin inhibits HCV NS5B polymerase activity, while its derivative silibinin reacts differently. The study highlights the need to account for the compositional differences between natural silymarin and its water-soluble forms in commercial products when interpreting the results (Bonifaz *et al.* 2009). Silymarin's hydrophobic properties are being investigated as a potential factor in its antiviral activity, as they may interact with lipid bilayers to stabilize membranes and prevent fusion. Additionally, the impact of silymarin on lipid metabolism is highlighted, particularly its suppression of the activity of the microsomal triglyceride transfer protein (MTP), the secretion of apolipoprotein B (apo B) and the generation of infectious virus particles. The authors suggest that silymarin's influence may extend beyond HCV and potentially affect liver diseases not caused by viruses (Li *et al.* 2018).

Despite variations observed between laboratory studies and clinical outcomes, the authors emphasize the need for further clinical and *in vitro* research to fully assess silymarin's hepatoprotective properties, metabolism and bioavailability. The following headings show the clinical trials that consider various delivery methods and preparations derived from silymarin. This comprehensive investigation not only elucidates the complex mechanisms of silymarin but also underscores its potential significance for liver disease research and therapeutic applications.

Alcoholic fatty liver disease

Alcoholic Fatty Liver Disease (AFLD) is a condition characterized by the accumulation of fat in liver cells due to excessive alcohol consumption (Armstrong and Guo 2019). While the exact cause of AFLD is not fully understood, several mechanisms contribute to its development, including decreased lipoprotein secretion, increased *de novo* lipogenesis, enhanced fatty acid uptake from circulation, and impaired fatty acid oxidation. Lipid peroxidation, oxidative stress, alcohol-induced inflammation, and acetaldehyde toxicity are mediators in these processes. AFLD can be reversed if alcohol consumption is avoided or moderated (Teschke 2018).

Cirrhosis, the advanced stage of alcohol-related liver disease (ARLD), differs from AFLD. Cirrhosis is characterized by irreversible fibrosis and scarring of liver tissue, resulting in liver dysfunction, as well as other complications such as hepatocellular carcinoma, ascites, portal hypertension, variceal hemorrhage and hepatic encephalopathy. Alcoholic hepatitis, an acute liver inflammation triggered by excessive alcohol intake, often precedes the development of cirrhosis after years of chronic alcohol abuse. Various factors influence the mortality rate of AFLD, including the quantity and duration of alcohol consumption, the presence of other liver diseases, nutritional status, and genetic predisposition. Without control or cessation of alcohol consumption, AFLD can progress to more severe forms of ARLD. Particularly in cases of severe alcoholic hepatitis and cirrhosis, the mortality rate from ARLD is significant. A study in the UK revealed that the 5-year survival rate for individuals with alcohol-related cirrhosis was 58%, whereas the rate for those with cirrhosis from other causes was 80% (Fan *et al.* 2019).

Effect of silymarin on AFLD

For centuries, hepatic ailments have been treated with silymarin, a complex mixture of polyphenolic compounds derived from milk thistle seeds. Thanks to its potent anti-inflammatory and antioxidant properties, silymarin can shield liver cells from alcohol-induced oxidative stress and cytotoxicity (Song *et al.* 2006). Additionally, silymarin can enhance the liver's capacity for regeneration by modifying enzymes associated with fibrosis, cirrhosis, and cellular damage (Kwon *et al.* 2013). Numerous studies have demonstrated that silymarin helps alleviate liver dysfunction, improve glycemic control, and inhibit the binding and absorption of toxins into liver cells. Moreover, silymarin can regulate the permeability of mitochondrial and cell membranes, thus preventing cell damage. In alcoholic fatty liver disease, silymarin primarily functions by stabilizing hepatocyte membranes and scavenging free radicals (Noel-Hudson *et al.* 1989; Akbari-Kordkheyli *et al.* 2019).

Kwon *et al.* (2013) conducted a randomized controlled trial to evaluate the impact of an herbal extract called silymarin MZ-80 on oxidative stress and collagen synthesis in patients with alcoholic liver cirrhosis. Sixty consecutive individuals diagnosed with this condition were randomized to receive either silymarin MZ-80 (150 mg three times a day) or a placebo for six months. Serum levels of amino-terminal propeptide of procollagen Type III (PIIINP), platelet malondialdehyde (MDA) and erythrocyte total glutathione (GSH) were assessed at the beginning and end of treatment.

PIIINP is a biomarker of collagen synthesis indicating fibrosis progression, MDA reflects oxidative stress as a lipid peroxidation marker, and GSH is an antioxidant protecting cells from oxidative damage (Taleb *et al.* 2018).

Compared to the placebo group, the treatment group showed significantly higher GSH levels and significantly lower MDA and PIIINP levels after treatment with silymarin MZ-80. These findings suggest that silymarin MZ-80 may benefit patients with alcoholic liver cirrhosis by reducing oxidative stress and inhibiting collagen production. However, routine liver tests such as blood transaminases, bilirubin, and albumin remained unchanged in both groups, indicating that these effects did not translate into improvements in these parameters. Further research is needed to confirm the therapeutic efficacy of silymarin MZ-80, but the authors noted that it is well-tolerated and may have some protective effects on liver function in individuals with alcoholic liver cirrhosis (Muzes *et al.* 1990; Wadhwa *et al.* 2022).

Conclusion

Silymarin, derived from milk thistle seeds, has been utilized in treating various liver disorders such as cirrhosis, Non-Alcoholic Fatty Liver Disease, Alcoholic Fatty Liver Disease, Drug-Induced Liver Injury, and viral hepatitis C. Its hepatoprotective, antioxidant, and anti-inflammatory properties position it as a potential therapeutic agent for these conditions. Clinical trials had demonstrated silymarin's efficacy in improving liver function, reducing mortality, and exhibiting anti-fibrotic properties. Moreover, it shielded the liver from oxidative injury induced by reactive oxygen species. In cases of DILI, silymarin shown its effectiveness by providing hepatoprotection and lowering the risk of liver injury. In viral hepatitis C, it displayed anti-inflammatory, antioxidant, and antiviral characteristics. Nevertheless, uncertainties persist regarding the optimal dosage, duration, and formulation for treating hepatitis C with silymarin.

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UA and MM: Designed the research and conducted the literature search. SMKR, AA, and MJ: Managed the data and wrote the first draft. MA and IA: Contributed to the literature search, and reviewed and edited the draft. MBA: Supervised the research, and reviewed, edited, and revised the final draft.

Conflicts of Interest

The authors declared no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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References

Abenavoli L, AA Izzo, N Milic, C Cicala, A Santini, R Capasso (2018). Milk thistle (*Silybum marianum*): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. *Phytother Res* 32:2202–2213

Abenavoli L, G Aviello, R Capasso, N Milic, F Capasso (2011). Milk thistle for treatment of nonalcoholic fatty liver disease. *Hepat Month* 11:173–177

Abenavoli L, R Capasso, N Milic, F Capasso (2010). Milk thistle in liver diseases: Past, present, future. *Phytother Res* 24:1423–1432

Adetuyi BO, FK Omolabi, PA Olajide, JK Oloke (2021). Pharmacological, biochemical and therapeutic potential of milk thistle (silymarin): A review. *World News Nat Sci* 37:75–91

Ahmed-Belkacem A, N Ahnou, L Barbotte, C Wychowski, C Pallier, R Brillet, JM Pawlotsky (2010). Silibinin and related compounds are direct inhibitors of hepatitis C virus RNA-dependent RNA polymerase. *Gastroenterology* 138:1112–1122

Akbari-Kordkheyli V, K Abbaszadeh-Goudarzi, M Nejati-Laskokalayeh, S Zarpou, A Khonakdar-Tarsi (2019). The protective effects of silymarin on ischemia-reperfusion injuries: A mechanistic review. *Iran J Basic Med Sci* 22:968–976

Alter MJ (2007). Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 13:2436–2441

Alvari A, FJ Ahmad, MZ Abdin (2012). Contemporary overview on clinical trials and future prospects of hepato-protective herbal medicines. *Rev Rec Clin Trials* 7:214–223

Armstrong LE, GL Guo (2019). Understanding environmental contaminants' direct effects on non-alcoholic fatty liver disease progression. *Curr Environ Health Rep* 6:95–104

Asrani SK, H Devarbhavi, J Eaton, PS Kamath (2019). The burden of liver diseases in the world. *J Hepatol* 70:151–171

Ball KR, KV Kowdley (2005). A review of Silybum marianum (milk thistle) as a treatment for alcoholic liver disease. *J Clin Gastroenterol* 39:520–528

Bijak M (2017). Silybin, a major bioactive component of milk thistle (*Silybum marianum* L. Gaernt.)—Chemistry, bioavailability, and metabolism. *Molecule* 22:1942

Bonifaz V, Y Shan, RW Lambrecht, SE Donohue, D Moschenross, HL Bonkovsky (2009). Effects of silymarin on hepatitis C virus and haem oxygenase-1 gene expression in human hepatoma cells. *Liver Intl* 29:366–373

Chalasani N, Z Younossi, JE Lavine, M Charlton, K Cusi, M Rinella, AJ Sanyal (2018). The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the american association for the study of liver diseases. *Hepatology* 67:328–357

Chambers CS, V Holeckova, L Petraskova, D Biedermann, K Valentov, M Buchta, V Kren (2017). The silymarin composition and why does it matter? *Food Res Intl* 100:339–353

Chen Z, R Yu, Y Xiong, F Du, SA Zhu (2017). A vicious circle between insulin resistance and inflammation in nonalcoholic fatty liver disease. *Lipids Health* 16:203-211

Child CG, JG Turcotte (1964). Surgery and portal hypertension. *Major Probl Clin Surg* 1:1–85

Diaz AA, JL Orejas, S Grumley, HP Nath, W Wang, WR Dolliver, RSJ Estepar (2023). Airway-occluding mucus plugs and mortality in patients with chronic obstructive pulmonary disease. *Jama* 329:1832–1839

El-Kamary SS, MD Shardell, M Abdel-Hamid, S Ismail, M El-Ateek, M Metwally, GT Strickland (2009). A randomized controlled trial to assess the safety and efficacy of silymarin on symptoms, signs, and biomarkers of acute hepatitis. *Phytomedicine* 16:391–400

Fan JG, L Wei, H Zhuang (2019). Guidelines for prevention and treatment of nonalcoholic fatty liver disease (2018, China). *J Digest Dis* 20:163–173

Feld JJ, JH Hoofnagle (2005). Mechanism of action of interferon and ribavirin in treatment of hepatitis C. *Nature* 436:967–972

Ferenci P (2016). Silymarin in the treatment of liver diseases: What is the clinical evidence? *Clin Liver Dis* 7:8–10

Freedman ND, TM Curto, C Morishima, LB Seeff, ZD Goodman, EC Wright, JE Everhart (2011). Silymarin use and liver disease progression in the Hepatitis C antiviral long-term treatment against cirrhosis trial. *Aliment Pharmacol Ther* 33:127–137

Gharagozloo M, E Velardi, S Bruscoli, M Agostini, M Di Sante, V Donato, C Riccardi (2010). Silymarin suppress CD4+ T cell activation and proliferation: Effects on NF-kappaB activity and IL-2 production. *Pharmacol Res* 61:405–409

Gillessen A, HH Schmidt (2020). Silymarin as supportive treatment in liver diseases: A narrative review. *Adv Ther* 37:1279–1301

Goldberg DS, KA Forde, DM Carbonari, JD Lewis, KB Leidl, KR Reddy, VL Re (2015). Population-representative incidence of drug-induced acute liver failure based on an analysis of an integrated health care system. *Gastroenterology* 148:1353–1361

Gu J, SJ Tang, SY Tan, Q Wu, X Zhang, CX Liu, HP Xiao (2015). An open-label randomized and multi-center clinical trial to evaluate the efficacy of Silibinin in preventing drug-induced liver injury. *Intl J Clin Exp Med* 8:4320–4327

Hackett E, D Twedt, D Gustafson (2013). Milk thistle and its derivative compounds: A review of opportunities for treatment of liver disease. *J Vet Intl Med* 27:10–16

Hashem A, Y Shastri, M Al Otaibi, E Buchel, H Saleh, R Ahmad, H Ahmed, F Al Idris, S Ahmed, M Guda (2021). Expert opinion on the management of Non-alcoholic fatty liver disease (NAFLD) in the Middle east with a focus on the use of silymarin. *Gastroenterol Insights* 12:155–165

Heleen K (2022). A review of regulatory compliance of medicines containing *Silybum marianum* (L.) Gaertn. (Milk Thistle) on the South African market. *Doctoral Dissertation*. University of Johannesburg, South Africa

Hossain N, P Kanwar, SR Mohanty (2016). A comprehensive updated review of pharmaceutical and nonpharmaceutical treatment for NAFLD. *Gastroenterol Res Pract* 2016:7109270

Iranikhah A, J Shapouri, A Heidari, M Aghaali, H Hajian (2017). Effects of silymarin on nonalcoholic fatty liver disease in children: A crossover clinical trial. *J Maz Univ Med Sci* 26:119–126

Jiang F, H Yan, L Liang, J Du, S Jin, S Yang, H Wang, T Hu, Y Zhu, G Wang (2021). Incidence and risk factors of anti-tuberculosis drug-induced liver injury (DILI): Large cohort study involving 4652 Chinese adult tuberculosis patients. *Liver Intl* 41:1565–1575

Khan I, H Ullah, I Ullah, F Bangash (2007). Study of the Physicochemical Properties of *Silybum marianum* Seed Oil. *J Chem Soc Pak* 29:545–548

Kheong CW, NR Nik Mustapha, S Mahadeva (2017). A randomized trial of silymarin for the treatment of nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 15:1940–1949

Kim M, SG Yang, JM Kim, JW Lee, YS Kim, JL Lee (2012). Silymarin suppresses hepatic stellate cell activation in a dietary rat model of non-alcoholic steatohepatitis: Analysis of isolated hepatic stellate cells. *Intl J Mol Med* 30:473–479

Koltai T, L Fliegel (2022). Role of silymarin in cancer treatment: Facts, hypotheses, and questions. *J Evid-Based Integr Med* 27:1–38

Kren V, D Walterova (2005). Silybin and silymarin-new effects and applications. *Biomed Papers* 149:29–41

Kwon DY, YS Jung, SJ Kim, YS Kim, DW Choi, YC Kim (2013). Alterations in sulfur amino acid metabolism in mice treated with silymarin: A novel mechanism of its action involved in enhancement of the antioxidant defense in liver. *Plant Med* 79:997–1002

Langmead L, DS Rampton (2001). Review article: Herbal treatment in gastrointestinal and liver disease-benefits and dangers. *Aliment Pharmacol Ther* 15:1239–1252

Li CC, CY Hsiang, SL Wu, TY Ho (2012). Identification of novel mechanisms of silymarin on the carbon tetrachloride-induced liver fibrosis in mice by nuclear factor-κB bioluminescent imaging-guided transcriptomic analysis. *Food Chem Toxicol* 50:1568–1575

Li H, MH Huang, JD Jiang, ZG Peng (2018). Hepatitis C: From inflammatory pathogenesis to anti-inflammatory/hepatoprotective therapy. *World J Gastroenterol* 24:5297–5311

Li S, HY Tan, N Wang, ZJ Zhang, L Lao, CW Wong, Y Feng (2015). The role of oxidative stress and antioxidants in liver diseases. *Intl J Mol Sci* 16:26087–26124

Luangchosiri C, A Thakkinstian, S Chitphuk, W Stitchantrakul, S Petraksa, AA Sobhonslidsuk (2015). A double-blinded randomized controlled trial of silymarin for the prevention of antituberculosis drug-induced liver injury. *BMC Complement Altern Med* 15:334

Luper S (1998). A review of plants used in the treatment of liver disease: Part 1. *Altern Med Rev* 3:410–421

Lv Y, S Gao, S Xu, G Du, J Zhou, J Chen (2017). Spatial organization of silybin biosynthesis in milk thistle [*Silybum marianum* (L.) Gaertn]. *Plant J* 92:995–1004

Mayer K, R Myers, S Lee (2005). Silymarin treatment of viral hepatitis: A systematic review. *J Viral Hep* 12:559–567

Muzes G, G Deak, I Lang, K Nekam, V Niederland, J Feher (1990). Effect of silimarin (Legalon) therapy on the antioxidant defense mechanism and lipid peroxidation in alcoholic liver disease (double blind protocol). *Orv Hetil* 131:863–866

Nasiri-Ansari N, E Kassi (2023). Special issue: Non-alcoholic fatty liver disease: From molecular basis to therapeutic advances. *Metab Open* 17:1–3

Noel-Hudson MS, CD Belilovsky, N Petit, A Lindenbaum, J Wepierre (1989). *In vitro* cytotoxic effects of enzymatically induced oxygen radicals in human fibroblasts: Experimental procedures and protection by radical scavengers. *Toxicol In Vitro* 3:103–109

Ore A, OA Akinloye (2019). Oxidative stress and antioxidant biomarkers in clinical and experimental models of non-alcoholic fatty liver disease. *Medicina* 55: 1–13

Pellicoro A, P Ramachandran, JP Iredale, JA Fallowfield (2014). Liver fibrosis and repair: Immune regulation of wound healing in a solid organ. *Nat Rev Immunol* 14:181–194

Pelt JFV, C Verslype, T Crabbe, Z Zaman, J Fevery (2003). Primary human hepatocytes are protected against prolonged and repeated exposure to ethanol by silibinin-dihemisuccinate. *Alcohol Alcohol* 38:411–414

Petta S, C Muratore, A Craxi (2009). Non-alcoholic fatty liver disease pathogenesis: The present and the future. *Dig Liver Dis* 41:615–625

Pradhan SC, C Girish (2006). Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. *Ind J Med Res* 124:491–504

Riaz T, M Akram, U Laila, MT Khalil, R Zainab, M Iftikhar, F Ahmet (2023). Milk thistle: A review of its phytochemistry, pharmacology and conventional uses. *Intl Arch Integr Med* 10:31–40

Saller R, J Melzer, J Reichling, R Brignoli, R Meier (2007). An updated systematic review of the pharmacology of silymarin. *Forsch Komplementmed* 14:70–80

Salvoza N, PJ Giraudi, C Tiribelli, N Rosso (2022). Natural compounds for counteracting nonalcoholic fatty liver disease (NAFLD): Advantages and limitations of the suggested candidates. *Intl J Mol Sci* 23:2764

Samee A, RM Amir, A Ahmad, FM Watto, M Ali, MT Azam, M Zahid (2023). Effectiveness of milk thistle on human body against diseases: A comprehensive review. *Sch Bull* 9:8–18

Seeff LB, HL Bonkovsky, VJ Navarro, G Wang (2015). Herbal products and the liver: A review of adverse effects and mechanisms. *Gastroenterology* 148:517–532

Shaker E, H Mahmoud, S Mnaa (2010). Silymarin, the antioxidant component and *Silybum marianum* extracts prevent liver damage. *Food Chem Toxicol* 48:803–806

Shi Z, J Wu, Q Yang, H Xia, M Deng, Y Yang (2020). Efficacy and safety of milk thistle preventive treatment of anti-tuberculosis drug-induced liver injury: A protocol for systematic review and meta-analysis. *Medicine* 99:e23674

Song Z, I Deaciuc, M Song, DY Lee, Y Liu, X Ji, C McClain (2006). Silymarin protects against acute ethanol-induced hepatotoxicity in mice. *Alcohol Clin Exp Res* 30:407–413

Sorrentino G, P Crispino, D Coppola, GD Stefano (2015). Efficacy of lifestyle changes in subjects with non-alcoholic liver steatosis and metabolic syndrome may be improved with an antioxidant nutraceutical: A controlled clinical study. *Drugs R D* 15:21–25

Surai PF (2015). Silymarin as a natural antioxidant: An overview of the current evidence and perspectives. *Antioxidants* 4:204–247

Taleb A, KA Ahmad, AU Ihsan, J Qu, N Lin, K Hezam, N Koju, L Hui, D Qilong (2018). Antioxidant effects and mechanism of silymarin in oxidative stress induced cardiovascular diseases. *Biomed Pharmacother* 102:689–698

Teschke R (2018). Alcoholic liver disease: Alcohol metabolism, cascade of molecular mechanisms, cellular targets, and clinical aspects. *Biomed* 6:106

Thrift AP, HB El-Serag, F Kanwal (2017). Global epidemiology and burden of HCV infection and HCV-related disease. *Nat Rev Gastroenterol Hepatol* 14:122–132

Tostmann A, MJ Boeree, RE Aarnoutse, WCD Lange, AJVD Ven, R Dekhuijzen (2008). Antituberculosis drug-induced hepatotoxicity: Concise up-to-date review. *J Gastroenterol Hepatol* 23:192–202

Trappoliere M, A Caligiuri, M Schmid, C Bertolani, P Failli, F Vizzutti, M Pinzani (2009). Silybin, a component of sylimarin, exerts anti-inflammatory and anti-fibrogenic effects on human hepatic stellate cells. *J Hepatol* 50:1102–1111

Wadhwa K, R Pahwa, M Kumar, S Kumar, PC Sharma, G Singh, R Verma, V Mittal, I Singh, D Kaushik (2022). Mechanistic insights into the pharmacological significance of silymarin. *Molecules* 27:5327

Wagoner J, A Negash, OJ Kane, LE Martinez, Y Nahmias, N Bourne, SJ Polyak (2010). Multiple effects of silymarin on the hepatitis C virus lifecycle. *Hepatology* 51:1912–1921

Wong RJ, A Ahmed (2014). Obesity and non-alcoholic fatty liver disease: Disparate associations among Asian populations. *World J Hepatol* 6:263–273

Wu S, Y Xia, X Lv, S Tang, Z Yang, Y Zhang, S Zhan (2015). Preventive use of hepatoprotectors yields limited efficacy on the liver toxicity of anti-tuberculosis agents in a large cohort of Chinese patients. *J Gastroenterol Hepatol* 30:540–545

Yao J, M Zhi, X Gao, P Hu, C Li, X Yang (2013). Effect, and the probable mechanisms of silibinin in regulating insulin resistance in the liver of rats with non-alcoholic fatty liver. *Braz J Med Biol Res* 46:270–277

Younossi ZM, AB Koenig, D Abdelatif, Y Fazel, L Henry, M Wymer (2016). Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatol* 64:73–84

Zhong T, Y Fan, XL Dong, X Guo, KH Wong, WT Wong, D He, S Liu (2021). An investigation of the risk factors associated with anti-tuberculosis drug-induced liver injury or abnormal liver functioning in 757 patients with pulmonary tuberculosis. *Front Pharmacol* 12:1-9