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A Comprehensive Review On The Pharmacology Of Milk Thistle (Silybum Marianum)

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ABSTRACT

Milk thistle (*Silybum marianum*), a medicinal herb with over 2,000 years of use, yields a potent flavonolignan mixture known as silymarin—whose major component is silybin (silibinin). This review synthesizes current knowledge on the plant's chemistry, pharmacokinetics, and pharmacodynamics, emphasizing its hepatoprotective actions—including anti-inflammatory, immunomodulatory, antifibrotic, antioxidant, and liver-regenerating effects. We also examine In-Vitro clinical trial data on conditions such as alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD), viral hepatitis, drug-induced liver toxicity, and mushroom poisoning. Though preclinical findings are promising, robust randomized controlled trials are needed to definitively establish clinical efficacy. Pharmacokinetically, silymarin has low oral bioavailability due to poor water solubility and rapid metabolism, though recent advancements in drug delivery systems—such as micellar formulations and nanoparticle encapsulations—have shown promise in enhancing absorption and systemic availability.

KEYWORDS

Milk thistle; *Silybum marianum*; silymarin; silybin; hepatoprotection; pharmacokinetics; flavonolignans; medicinal plants; antioxidant; anti-inflammatory; liver disease; cultivation; phytochemistry; future therapeutics.

INTRODUCTION

For centuries, herbal remedies have played a central role in traditional medicine systems around the world, including Chinese, Japanese (Kampo and Hozai), Ayurvedic, African, Amazonian, and Arabic practices. Many of these natural formulations are considered safe and effective, with numerous plant species having undergone scientific evaluation that led to the development of clinically approved herbal drugs. However, a substantial number of medicinal plants—both widely known and relatively obscure—remain underexplored and require in-depth pharmacological and clinical investigation [1,2].

One such plant of significant interest is *Silybum marianum* Gaertn., commonly referred to as milk thistle. Belonging to the Asteraceae family, this species is indigenous to the Mediterranean region and has a long history of cultivation across Europe. In India, it grows predominantly in the higher elevations of Jammu and Kashmir, typically between 1,800 and 2,400 meters above sea level, and is also cultivated ornamentally in various other environments [3]. Historical records indicate that its medicinal use dates back to the 4th century BCE, as mentioned by Theophrastus. Later, in the 1st century CE, Dioscorides, a Greek physician serving in the Roman military, categorized several edible thistles under the genus *Silybum* [4].

Traditionally, milk thistle has been employed in the treatment of various disorders, particularly those involving hepatic dysfunction. The primary bioactive compound derived from this plant is silymarin, a complex mixture of flavonolignans such as silibinin (also known as silybin), silychristin, and silidianin. Experimental pharmacological studies have shown that silymarin exhibits notable antioxidant, anti-inflammatory, and antifibrotic activities. Despite mixed results in clinical studies, milk thistle has shown potential in the management of liver-related conditions, including hepatitis B and C, alcoholic liver disease, cirrhosis, and non-alcoholic fatty liver disease (NAFLD) [5].

Silymarin's longstanding safety record and low incidence of adverse effects support its continued use as a complementary therapeutic agent. However, its clinical effectiveness is hampered by poor water solubility and low oral bioavailability. Recent advances in drug delivery technologies—such as liposomal carriers, phytosomes, and nanoparticles—have been investigated to enhance its absorption and therapeutic efficacy.

This review aims to consolidate existing literature on the pharmacological activities, chemical constituents, and pharmacokinetic properties of *Silybum marianum*, with a focus on its therapeutic potential in liver diseases and other pathological conditions including cancer, viral infections, inflammation, fibrosis, and tumorigenesis. Additionally, this article briefly discusses safety profiles, adverse effects, and formulation strategies designed to overcome the limitations associated with silymarin administration [6,7].

Fig. 1[2]: Milk thistle (Silybummarianum) flower, leaves, seeds.

BOTANICAL DESCRIPTIONS

Milk thistle (*Silybum marianum*) is a robust, erect annual or biennial herbaceous plant, typically growing between 1.5 to 3 meters (5 to 10 feet) in height. It is characterized by its stout, spiny stems and large, glossy green leaves that are deeply lobed or pinnatifid, with pronounced white veins and spiny margins. These distinctive white veins give the foliage a marbled appearance and contribute to its fibrous texture. When damaged, the plant exudes a milky latex from its leaves and stems—a trait that inspired its common name [2].

The plant forms a basal rosette of leaves, from which flowering stems emerge. Flowering occurs between June and September. During this period, each stem typically produces a solitary, terminal flower head that is large, slightly aromatic, and purple in color. The flower heads are surrounded by spiny bracts and display ridged structures that end in sharp points, enhancing their protective morphology.

Following pollination, the plant produces dry fruits known as achenes, which are approximately 6–7 mm in length. These achenes are dark-colored, transversely wrinkled, and often display grey speckles with a yellow ring at the apex. Each achene is equipped with a long, white pappus that facilitates seed dispersal. The mature fruits are generally glossy, varying in color from brown to grey, and often display distinctive spotting [4].

Fig. 2[8]: Milk thistle (Silybum marianum) flowering head.

TRADITIONAL CULTIVATION AND USAGE

Historically, *Silybum marianum* was cultivated throughout Europe not only for its medicinal properties but also as a vegetable crop. The leaves, once stripped of their spines, were consumed raw in salads or cooked like spinach. Additionally, the stalks, roots, and flower heads were edible, and the roasted seeds were often used as a coffee substitute. The medicinal application of milk thistle dates back as early as the 4th century BCE, with the earliest recorded mention by Theophrastus. In traditional European herbal medicine, the seeds were used as a galactagogue to enhance milk production in lactating women and served a variety of other purposes including treatment for liver-related conditions such as gallstones, dyspepsia, and splenic congestion [8]. They were also used for circulatory disorders (e.g., varicose veins), metabolic conditions such as diabetes, and gynecological issues including amenorrhea, uterine bleeding, and menstrual irregularities. The protective effects of milk thistle on liver function were recognized as early as the first century CE by Greek and Roman physicians, underscoring its long-standing role in traditional hepatoprotective therapies [9,10].

PRESENT DAY CULTIVATION AND USAGE

Milk thistle is native to regions including Asia Minor, North Africa, Southern Europe, Southern Russia, and the Kashmir region of India. Today, it has been naturalized and cultivated across much of Europe, North and South America, China, Australia, and Canada. It is typically grown in dry, rocky soils, both for its medicinal value and its ornamental appeal due to its distinctive variegated foliage. Harvesting of mature seeds generally occurs by the end of July [2].

In modern herbal medicine, milk thistle is predominantly utilized for its hepatoprotective properties. Its seeds and standardized extracts are widely used to manage liver disorders and toxic liver damage, particularly in cases involving exposure to industrial toxins or poisonous mushrooms. It is frequently prescribed for chronic hepatic conditions such as hepatitis, jaundice, alcoholic liver disease, hepatic fibrosis, cirrhosis, and non-

alcoholic fatty liver disease. Additionally, milk thistle is a common ingredient in nutraceuticals aimed at liver health [8,9].

In homeopathic practice, tinctures prepared from the seeds have traditionally been employed in the treatment of conditions such as jaundice, gallstones, varicose veins, peritonitis, hemorrhagic disorders, and other liver-related ailments. Commercial formulations are now available globally, most commonly in the form of capsules or tablets containing standardized extracts of milk thistle seed or silymarin [10].

CHEMICAL COMPOSITION

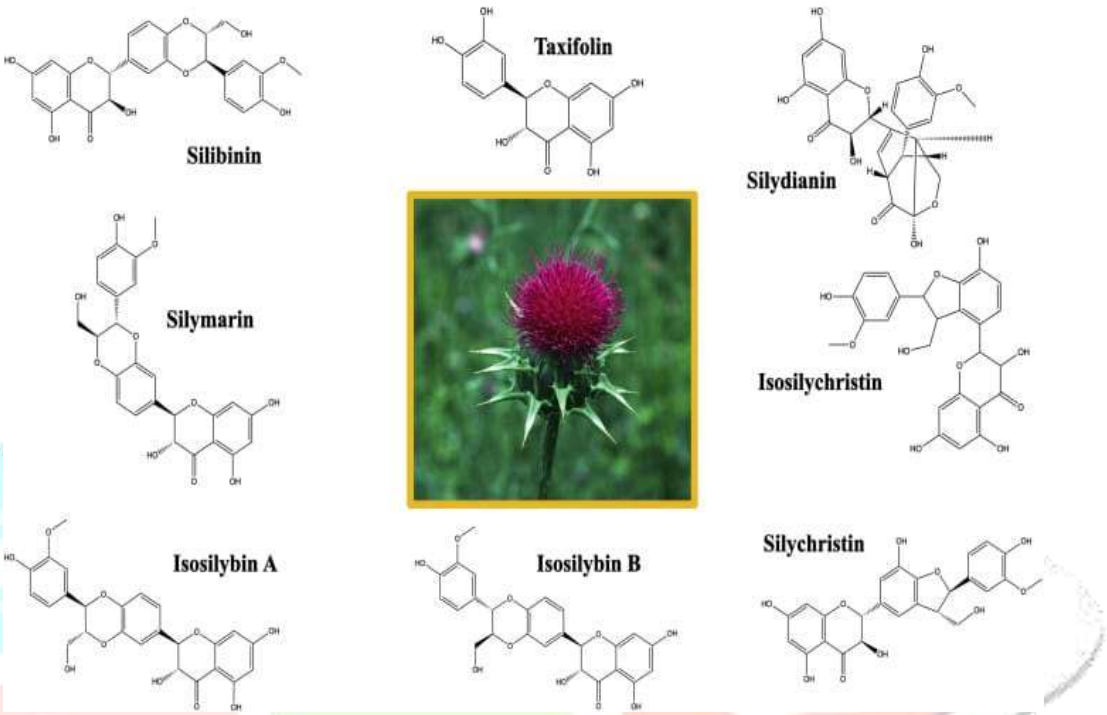

Fig. 3[2]: Chemical structures of Milk thistle constituents.

The primary bioactive constituents of *Silybum marianum* (milk thistle) are a group of flavonolignans collectively known as silymarin, which accounts for approximately 70–80% of the total extract obtained from the plant's seeds, fruits, and, to a lesser extent, leaves [1]. The empirical formula of silymarin is $C_{25}H_{22}O_{10}$, and it comprises several structurally related flavonolignans, with silybin (also called silibinin) being the most abundant and pharmacologically significant [2].

Silymarin is a chemically complex mixture, primarily composed of four major flavonolignans: silybin, silychristin, silydianin, and isosilybin. Among these, silybin exists in two diastereomeric forms, silybin A and silybin B, as does isosilybin, yielding isosilybin A and isosilybin B [11,12]. These diastereomers contribute to the overall stereochemical diversity and may differentially influence the biological activity of the extract.

In addition to flavonolignans, silymarin contains minor flavonoid components such as taxifolin, quercetin, and apigenin, which may synergize with the primary constituents to enhance therapeutic efficacy [13,14].

The pharmacological properties of milk thistle, particularly its hepatoprotective, antioxidant, and anti-inflammatory effects, are largely attributed to silybin, making it the most studied and therapeutically relevant component of the extract [15,16]. The structural complexity of silymarin, especially due to the presence of multiple diastereomers, adds a layer of biochemical variability that can influence both pharmacokinetics and pharmacodynamics, and is a factor of growing interest in ongoing pharmacological research [17].

PHARMACOKINETICS OF SILYMARIN

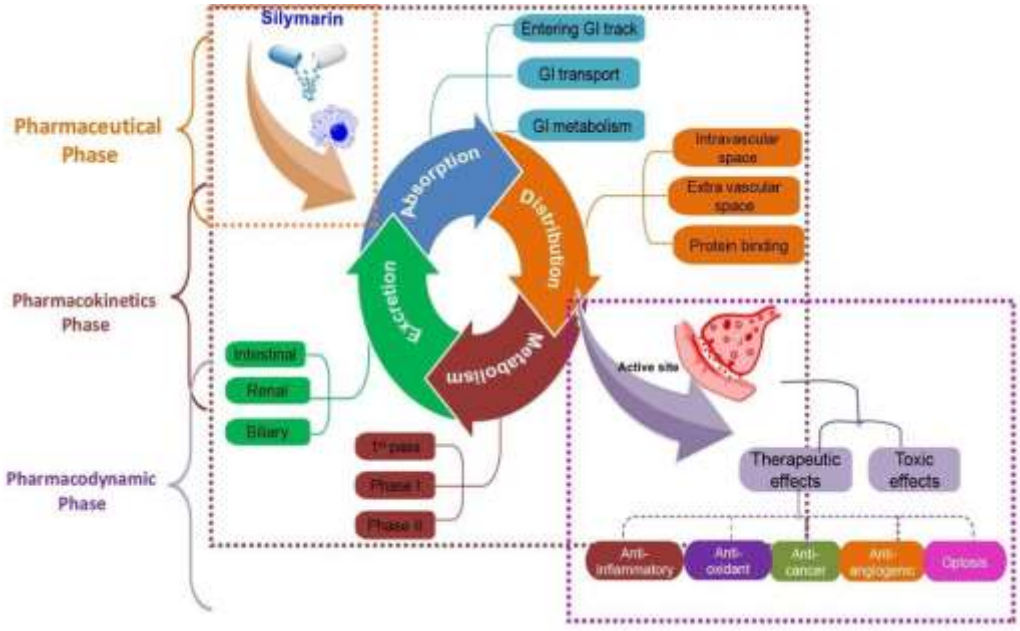

Fig. 4[2]: Pharmacokinetics of Silymarin

Pharmacokinetics (PK) refers to the study of how a compound is absorbed, distributed, metabolized, and eliminated within the body [1,18]. Understanding these processes is essential for optimizing therapeutic efficacy, determining appropriate dosing regimens, and evaluating systemic exposure to bioactive compounds [19,20]. In the case of *Silybum marianum*, silybin is the principal bioactive molecule in silymarin and is considered the main pharmacologically active constituent [21,22].

Absorption

Silymarin has inherently low water solubility, which significantly limits its oral bioavailability. Preclinical studies, including in vitro and in vivo models, indicate that silymarin can be absorbed by intestinal epithelial cells; however, the extent of this absorption is limited [2]. Following oral administration of powdered extracts, peak plasma concentrations of silybin are typically low—approximately 20 ng/mL within 30 minutes. The bioavailability of silymarin upon oral intake ranges between 23% and 47%. Peak plasma levels (C_{max}) are generally observed between 6 to 8 hours post-administration. Due to these limitations, silymarin is often administered in encapsulated or enhanced formulations (e.g., phytosomes, nanoparticles) to improve solubility and absorption [23,24].

Distribution

Once absorbed, silybin is rapidly distributed across various tissues, both in its free and conjugated forms [25,26]. Animal studies have demonstrated that tissue concentrations peak within one hour of administration at doses of 50 mg/kg. The compound has been detected in multiple organs including the liver, lungs, stomach, skin, pancreas, and prostate [27]. For example, liver tissue concentrations reached 8.8 ± 1.6 µg/g, while concentrations in the pancreas were reported at 5.79 ± 1.10 µg/g. Silybin also exhibits moderate to high plasma protein binding, ranging from approximately 46% to 70.3% in rat models [28].

Metabolism

Silybin undergoes extensive biotransformation primarily via phase II conjugation pathways, which include glucuronidation and sulfation. These reactions play a significant role in the compound's metabolic clearance. In vitro studies using human hepatocytes have revealed that silybin is converted into a range of metabolites, including one major demethylated product, three mono-hydroxylated metabolites, and one di-hydroxylated metabolite. This metabolic complexity contributes to the variability in its systemic bioavailability and therapeutic performance [25].

Elimination

Elimination of silymarin from the body is relatively rapid. Less than 3% of an orally administered dose is recovered in urine, indicating poor renal excretion. In fact, only 1–2% of orally administered silybin is excreted unchanged in the urine within 24 hours. The liver and biliary system serve as the principal routes for elimination, with silybin concentrations in bile reported to be up to 100-fold higher than in plasma. This suggests that biliary excretion is the dominant pathway for silybin clearance [29].

MEDICINAL PROPERTIES

1) ANTI-INFLAMMATORY POTENTIAL OF SILYMARIN

Recent research has brought growing attention to silymarin's anti-inflammatory activity, expanding its therapeutic relevance beyond its established hepatoprotective effects. Chronic inflammation is intricately linked to the pathophysiology of numerous diseases, often creating a feedback loop that intensifies both inflammatory processes and disease progression. This complexity presents significant challenges for long-term management using conventional anti-inflammatory drugs.

Traditional pharmacological treatments for inflammation—such as aspirin, ibuprofen, naproxen, and indomethacin—primarily act by inhibiting cyclooxygenase (COX) enzymes involved in the biosynthesis of prostaglandins (PGs), which mediate fever, pain, and inflammatory responses. While effective, these agents frequently lead to adverse side effects, notably gastrointestinal irritation, which may result in more serious complications over extended use [30].

MECHANISMS OF ANTI-INFLAMMATORY ACTION

Silymarin has demonstrated anti-inflammatory effects through several key molecular mechanisms, including:

1. **Inhibition of pro-inflammatory cytokine secretion** – Silymarin reduces the expression and release of inflammatory mediators such as TNF-α, IL-1β, and IL-6 [31].
2. **Suppression of the NF-κB signaling pathway** – This transcription factor plays a pivotal role in regulating inflammation, and silymarin has been shown to inhibit its activation, thus downregulating inflammatory gene expression [32].
3. **Modulation of the MAPK signaling pathway** – By interfering with mitogen-activated protein kinases (MAPKs), silymarin helps to regulate cellular responses to stress and inflammation [33].
4. **Activation of antioxidant defense systems** – Silymarin contributes to the enhancement of endogenous antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase), reducing oxidative stress, which is often a driver of inflammation [34].

CLINICAL APPLICATIONS AND THERAPEUTIC POTENTIAL

The anti-inflammatory activity of silymarin has been substantiated through a range of preclinical and in vitro studies [35]. These findings have laid the groundwork for clinical investigations into its potential therapeutic role in human diseases characterized by inflammation and oxidative damage [36].

Ongoing and emerging clinical research has evaluated silymarin in a variety of conditions, including:

- **Radiation-induced mucositis and dermatitis**
- **Cutaneous inflammatory disorders**
- **Diabetes mellitus and its complications**
- **β-thalassemia major**
- **Oxidative stress-related diseases**

Furthermore, silymarin is being explored as a potential therapeutic or adjunctive treatment for non-alcoholic fatty liver disease (NAFLD), inflammatory bowel disease (IBD), rheumatoid arthritis, and chronic dermatological conditions such as psoriasis and eczema [37].

Although early clinical findings are promising, more extensive randomized controlled trials (RCTs) are essential to fully establish silymarin's clinical efficacy, optimal dosing strategies, treatment duration, and safety profile. Despite these gaps, silymarin remains a strong candidate for use as a complementary therapy in a broad spectrum of inflammation-mediated diseases [38,39].

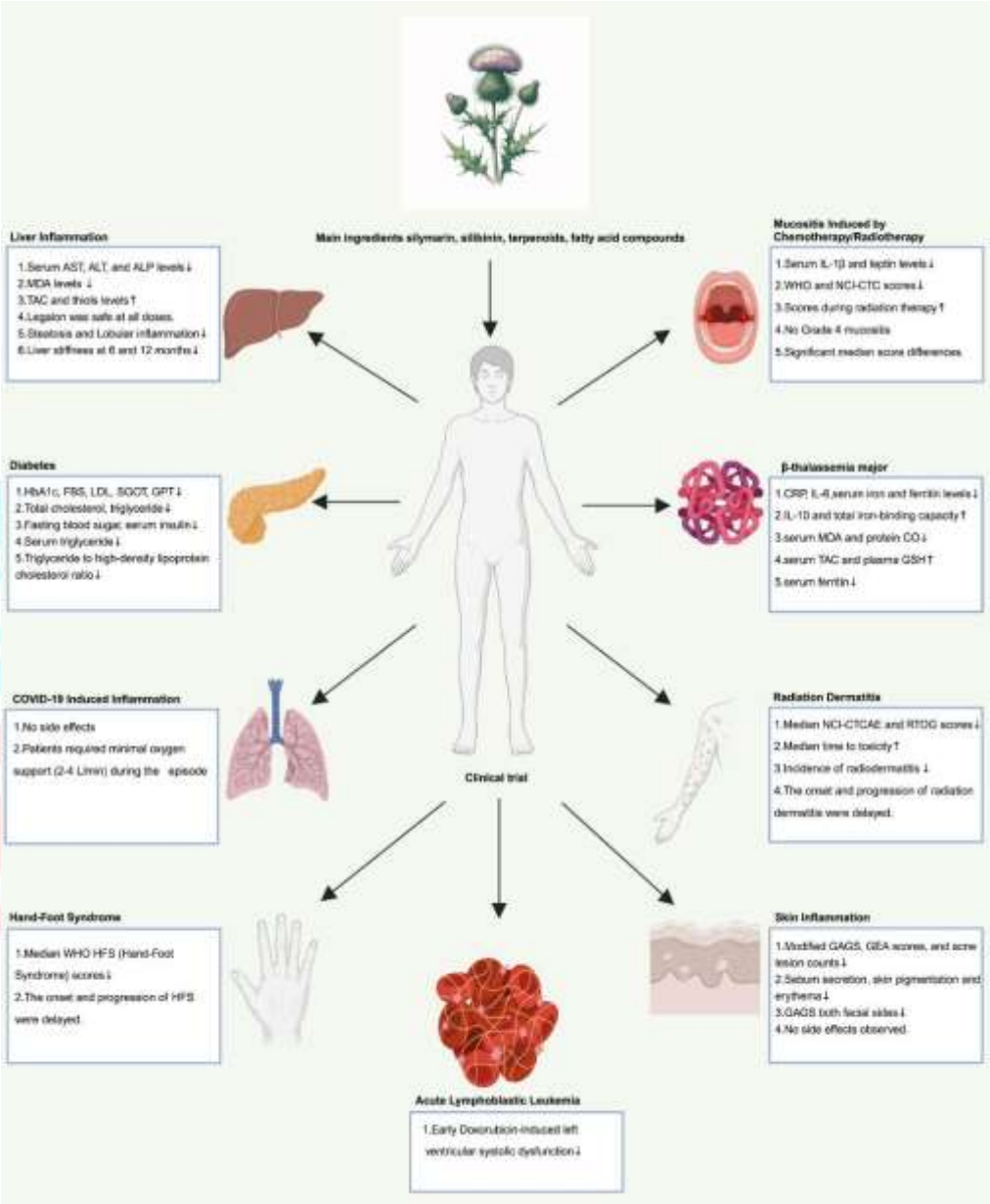

Fig. 5[30]: Clinical applications of silymarin as an anti-inflammatory agent

2) ANTIOXIDANT PROPERTIES OF SILYMARIN

Silybum marianum (milk thistle) has a long-standing history of use in traditional medicine, particularly for the treatment of hepatic and biliary disorders. One of the key mechanisms underlying its therapeutic effects is its potent antioxidant activity. Recent investigations have aimed to evaluate silymarin's ability to scavenge free radicals and protect biological systems against oxidative damage through a series of well-established in vitro assays.

Experimental Assessment of Antioxidant Activity

In vitro antioxidant properties of silymarin have been characterized using the following methodologies:

1. **Ferric Reducing Antioxidant Power (FRAP) Assay** – to evaluate total antioxidant capacity based on the reduction of ferric ions.
2. **2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay** – a common assay used to determine the free radical neutralizing potential of antioxidants.
3. **Inhibition of Red Blood Cell (RBC) Hemolysis** – using peroxyl radicals generated from 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) to assess protection of erythrocyte membranes against oxidative stress.
4. **Inhibition of Plasma Oxidation** – using copper ions (Cu^{2+}) to induce lipid peroxidation in plasma, simulating oxidative conditions in the circulatory system.

Additionally, the total phenolic content of silymarin was quantified using the Folin–Ciocalteu method, with results expressed in gallic acid equivalents (GAE). The phenolic content of silymarin was determined to be 0.484 ± 0.017 mg GAE/mg, which was notably higher than that of green tea extract (0.313 ± 0.095 mg GAE/mg), a well-known antioxidant benchmark [40].

Interpretation and Implications

All assays indicated that silymarin's antioxidant effects were concentration-dependent, confirming its efficacy in scavenging free radicals and protecting cellular components from oxidative injury. These findings support the hypothesis that silymarin exerts its biological protective effects, in part, through its ability to neutralize reactive oxygen species (ROS) and maintain redox homeostasis.

Given its high phenolic content and demonstrated antioxidant potency, silymarin represents a promising natural compound for dietary supplementation aimed at preventing or mitigating diseases associated with oxidative stress, including chronic inflammation, metabolic disorders, cardiovascular diseases, and degenerative conditions [41].

3) HEPATOPROTECTIVE EFFECTS OF SILYMARIN

Silymarin, the principal active complex derived from *Silybum marianum*, has been extensively studied for its hepatoprotective properties. Its therapeutic role in liver diseases is supported by a wide range of preclinical and clinical evidence [42]. Other botanicals, such as *Phyllanthus niruri* and *Panus giganteus (Berk.)*, have also shown hepatoprotective potential; however, silymarin remains one of the most widely studied and utilized plant-derived agents in liver protection [43].

Silymarin contains at least seven distinct flavonolignans, along with the flavonoid taxifolin, which together contribute to its potent antioxidant and hepatoprotective actions. Its protective effects are largely attributed to its ability to neutralize free radicals generated during the hepatic metabolism of toxic substances such as ethanol, acetaminophen, and carbon tetrachloride (CCl_4). These free radicals can initiate lipid peroxidation, disrupt cell membranes, and damage hepatocyte function [44].

One of the key mechanisms through which silymarin exerts its hepatoprotective effects is by increasing intracellular levels of glutathione (GSH) in liver cells. Glutathione is a crucial antioxidant molecule involved in detoxification and the maintenance of cellular redox balance. By enhancing GSH levels, silymarin strengthens the liver's natural antioxidant defenses.

Additionally, evidence suggests that silymarin stimulates protein synthesis in hepatocytes, which may support liver regeneration and repair [45,46]. This action is thought to occur via activation of RNA polymerase I, resulting in increased ribosomal RNA production and enhanced protein biosynthesis in isolated liver cells.

Clinical data also support its potential benefits in liver disease. In one human study, patients with alcoholic cirrhosis who received silymarin showed a modest but statistically significant improvement in survival rates

compared to untreated controls, indicating its possible role in slowing disease progression and improving long-term outcomes [47,48].

4) EFFECT ON ALCOHOLIC LIVER DISEASE

The metabolism of ethanol is directly involved in the generation of reactive oxygen species and reactive nitrogen species that create an environment conducive to oxidative stress [49]. Silymarin, an antioxidant, provided effective resistance to alcoholic cirrhosis in baboons. We have also found whether through silibin effective in alcoholic cirrhosis or through some other means of protecting rats from ethanol enhanced liver oxidative stress that SDH was effective when using human hepatocytes exposed to ethanol in vitro [50.51]. It is reported that when given silymarin, patients with alcoholic liver disease had serum bilirubin, aspartate aminotransferase and alanine aminotransferase return to normal levels, while serum gamma-glutamyl transferase activity and procollagen III peptide declined [52,53]. Despite these results, in a study by Stickel et al (2003) silymarin did not demonstrate clinically significant efficacy in alcoholic cirrhosis and others have suggested that silymarin had no impact on survival in patients with liver cirrhosis and alcohol abuse [54].

5) ANTI-CANCER ACTIVITY OF SILYMARIN

Silymarin has demonstrated significant anti-cancer properties across various experimental models, showing promise as both a chemopreventive and therapeutic agent. Supplementation with silymarin has been reported to markedly inhibit tumor growth and even induce regression of established tumors, with more pronounced effects observed in early-stage (stage I) tumors. The underlying mechanisms appear to involve the suppression of promoter-induced processes such as edema, hyperplasia, increased cell proliferation, and oxidative stress [56].

In vivo studies highlight silymarin's ability to exert anti-proliferative, pro-apoptotic, and anti-angiogenic effects, particularly noted in prostate cancer models. For example, when administered during the promotion phase of 4-nitroquinoline 1-oxide (4-NQO)-induced carcinogenesis in rats, silymarin demonstrated chemopreventive activity against tongue squamous cell carcinoma. This effect is believed to result from the modulation of key enzyme activities, reduction in cell proliferation rates, and alterations in prostaglandin E2 (PGE_2) levels [57].

Further research has extended these findings to long-term tumorigenesis models and various human cancer cell lines, including prostate, breast, and cervical carcinomas [58]. Treatment with silibinin, a major active component of silymarin, significantly inhibited cell growth and DNA synthesis in a time-dependent manner. Notably, cervical carcinoma cells showed a marked loss of viability following silibinin exposure, underscoring its potent cytotoxic effects in certain cancer types [59].

Collectively, these studies support silymarin's potential role as a multi-targeted anti-cancer agent, capable of interfering with tumor initiation, promotion, and progression through diverse molecular pathways [60].

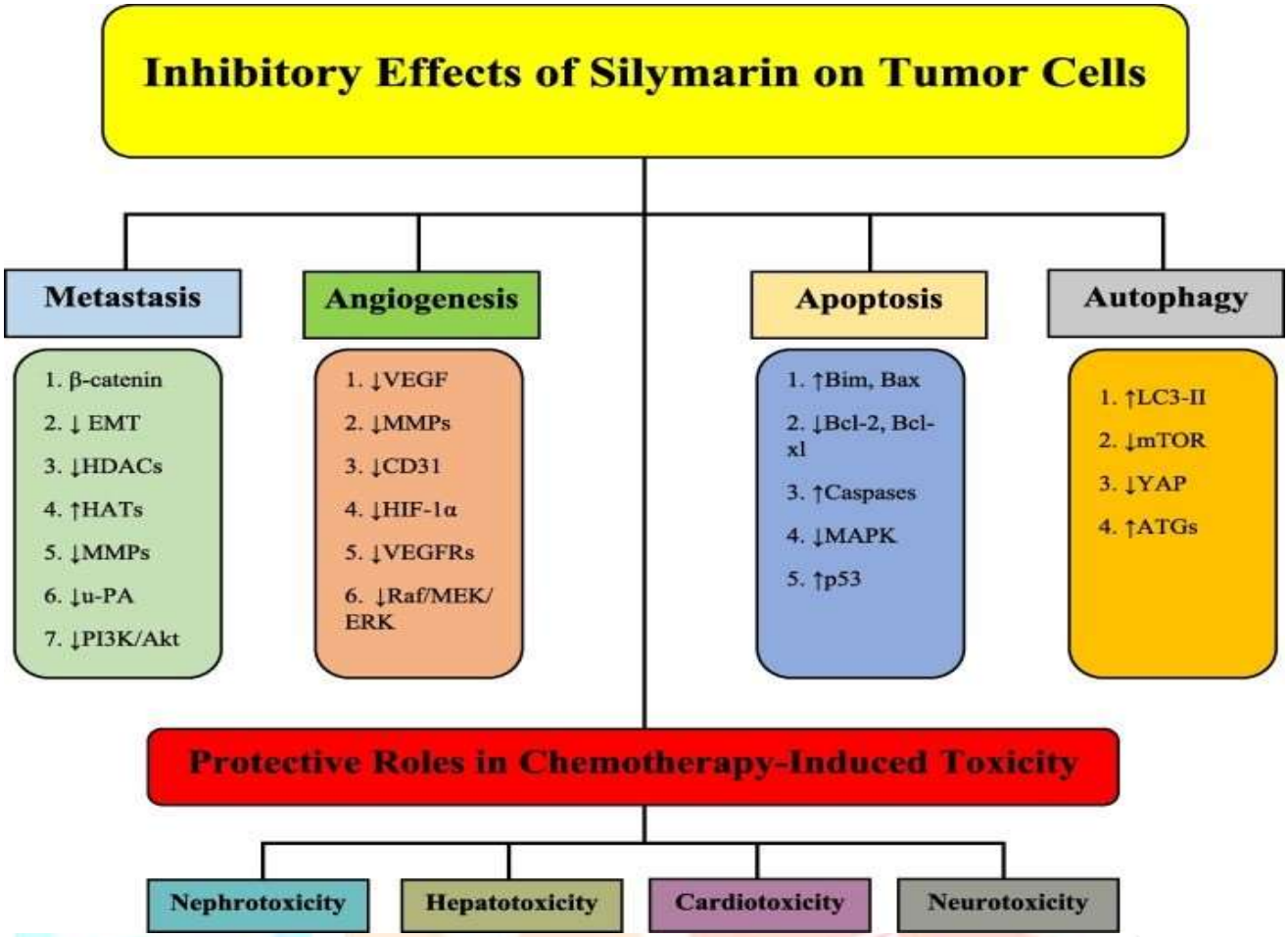

Fig. 6[55]: Anti-tumor effects of silymarin, its molecular and signaling targets, and protective roles in chemotherapy-induced toxicity. EMT, epithelial-mesenchymal transition; HDACs, histone deacetylases; HATs, histone acetyltransferases; MMPs, matrix metalloproteinases; u-PA, urokinase plasminogen activator; VEGF, vascular endothelial growth factor; HIF-1α, hypoxia-inducible factor 1 alpha.

6) IMMUNOMODULATORY ACTIVITY

Treatment of mice with silymarin intraperitoneally with the endotoxin-free neutralizing anti-IL-12 antibody negated the protective actions silymarin had against the UVB suppression of the contact hypersensitivity response. Furthermore, treatment with silymarin did not protect against the UVB suppression of the contact hypersensitivity response in IL-12 knockout mice either while it did protect against this suppression in their wild-type mice. Furthermore, i.p. injection of IL-12 to IL-12 knockout mice that had been treated with silymarin or IL-12 knockout mice that had not been treated with silymarin resulted in enhancement to the contact hypersensitivity response when compared to the response in either mice exposed to UVB alone or silymarin + UVB. This indicates that silymarin could protect mice from UVB-mediated immunosuppression and this protection is mediated in part through IL-12 [1,57].

7) ANTI-VIRAL EFFECTS OF SILYMARIN

Silymarin has shown promising effects in managing viral hepatitis, not by directly inhibiting viral replication, but through its ability to modulate the inflammatory and cytotoxic cascades triggered by viral infections. Specifically, silymarin suppresses nuclear factor kappa B (NF-κB)-dependent transcription in human hepatoma cells and reduces tumor necrosis factor-alpha (TNF-α) expression in activated peripheral blood mononuclear cells. These anti-inflammatory and antiviral actions may offer therapeutic benefits for patients with chronic hepatitis C [61].

In cellular models, silybin and its derivative dehydrosilybin significantly reduce inducible cytochrome P450 1A1 (CYP1A1) activity in both human keratinocyte (HaCaT) and hepatoma (HepG2) cells, with stronger effects observed in HaCaT cells. Silybin also inhibits the growth of HepG2 cells (hepatitis B virus-negative

with intact p53) and Hep38 cells (hepatitis B virus-positive with mutated p53), inducing apoptosis and cell cycle arrest. In Hep3B cells, silybin causes arrest at the G1 and G2/M phases, accompanied by modulation of key regulatory proteins, including an increase in p21/p27 and a decrease in cyclins D1, D3, E, and cyclin-dependent kinases (CDK2, CDK4). It also inhibits kinase activities related to CDK2, CDK4, and CDC2 in hepatocellular carcinoma (HCC) cells, contributing to its cytostatic and cytotoxic effects [62].

Intravenous Silibinin and Its Clinical Use

Silibinin, a primary component of silymarin consisting of silybin A and B, exhibits multiple biological activities, including antioxidant, immunomodulatory, antiproliferative, antifibrotic, and antiviral properties. Both silymarin and silibinin block hepatitis C virus (HCV) infection at various stages, such as viral entry, fusion, RNA and protein synthesis, NS5B RNA-dependent RNA polymerase activity, and viral transmission in cell culture [63].

The intravenous formulation of silibinin, marketed as Legalon SIL® (silibinin-C-2',3-dihydrogen succinate disodium salt), has been used in several clinical scenarios, although controlled double-blind studies are lacking. Key clinical contexts include:

- **Treatment of Non-Responders to Pegylated Interferon and Ribavirin:** In patients unresponsive to conventional therapy, intravenous silibinin induced a dose-dependent decline in HCV RNA levels within seven days. Combination therapy including silibinin resulted in sustained virologic response in a subset of patients.
- **Treatment of "On-Treatment" Non-Responders:** Exploratory studies have investigated intravenous silibinin in patients not responding to standard antiviral therapy, demonstrating potential benefits.
- **Use in Liver Transplantation:** Silibinin has been applied to prevent graft reinfection in liver transplant recipients with hepatitis C, with mixed success. It has also been used to treat acute liver failure caused by Amanita phalloides poisoning.

Mechanisms Underlying Silibinin's Antiviral Activity

Legalon SIL® retains the antiviral properties of silibinin, inhibiting HCV fusion, replication, and production of infectious virus particles. Both Legalon SIL® and silibinin inhibit the NS5B polymerase in vitro. Recent studies suggest that silibinin impairs HCV entry by disrupting clathrin-mediated endocytosis, interfering with viral trafficking inside the host cell. Resistance mutations in the NS4B protein of HCV have been identified, partially conferring resistance to Legalon SIL®, highlighting the complex interaction between silibinin and viral components [64].

Current Clinical Trials

Silymarin is actively being investigated in multiple clinical trials worldwide. Ongoing studies include:

- A randomized, placebo-controlled trial evaluating silymarin's safety and efficacy in acute viral hepatitis (University of Maryland).
- An observational study on the combined effects of silymarin and green tea extract in chronic hepatitis C patients (University of North Carolina).
- Clinical evaluation of Legalon SIL® for preventing HCV recurrence post-liver transplantation (Italy).
- Investigation of Legalon SIL® in the treatment of mushroom poisoning (USA).

These trials aim to further elucidate the therapeutic potential and optimize the clinical use of silymarin and its components in viral infections [65].

8) ANTI- FIBROTIC ACTIVITY

The detrimental role of hepatic stellate cells and their myofibroblast derivatives is integral to liver fibrogenesis. Silibinin at a concentration of 10'mol/l inhibited proliferation of freshly isolated rat hepatic stellate cells but this was not apparent in their viability, morphology or cytoskeletal organization [1]. Further, it inhibited transformation of myofibroblasts and down-regulated the expression of the profibrogenic

transforming growth beta (TGF-B) and extracellular matrix component genes. Hepatoprotective effects may be linked to alterations in TGFB1 and c-myc expression in the liver. Inhibition of hepatic stellate cell transition and proliferation may comprise one/important aspect of potential antifibrotic properties [66,67].

9) EFFECT ON GROWTH FACTORS

Milk thistle has also enhanced nerve growth factor-induced neurite outgrowth in PC 12 neural cells and increased their survival in culture. Further, it has shown the ability to decrease oxidative stress-induced cell death in cultured rat hippocampal neurons, and thus can promote neuronal differentiation and survival. Silibinin, even at pharmacologically achievable concentrations (0.02-20 mM), acts as an antiproliferative agent. Silibinin increased insulin-like growth factor-binding protein 3 (IGFBP-3) accumulation in PC-3 cell conditioned medium, and there was also a dose-dependent increase of IGFBP-3 mRNA abundance [68]. Additionally, we used an IGFBP-3 antisense oligodeoxynucleotide that inhibited silibinin-induced IGFBP-3 gene expression and protein accumulation, which resulted in a reduction of the antiproliferative actions of silibinin. Silibinin resulted in reduced insulin receptor substrate 1, tyrosine phosphorylation, indicating an inhibitory action on the insulin-like growth factor I receptor-mediated signaling pathways [69].

10) GLYCAEMIC AND LIPIDAEMIC CONTROL

Milk thistle, particularly its active compound silibin (also known as silybin or silibinin), has emerged as a promising agent in managing insulin resistance and hyperglycemia associated with diabetes. One key pathological mechanism in diabetic complications involves the accumulation of sorbitol within cells of insulin-independent tissues under hyperglycemic conditions. This process, facilitated by the enzyme aldose reductase, leads to cellular water retention and tissue damage. Silibin's ability to inhibit aldose reductase positions it as a potential therapeutic candidate to prevent and treat diabetes-related complications such as cataracts and neuropathy [70].

Anti-Obesity and Anti-Diabetic Properties of Silibin

In Vitro Studies: Silibin's aldose reductase inhibition has been extensively studied in various tissues—including the testis, placenta, nervous system, lens of the eye, and pancreatic islets—that rely on insulin-independent glucose transport. For example, in models simulating diabetic neuropathy, treatment with silibin at nanomolar concentrations (10 nM) preserved Na-K-ATPase activity and reduced glucose-induced mono-ADP-ribosylation loss in SY5Y neuroblastoma cells. Notably, similar effects were absent when fructose or galactose was used instead of glucose, underscoring silibin's glucose-specific protective properties [71].

Ex Vivo Animal Studies: Silibin has demonstrated an inhibitory effect on hepatic glycolysis by suppressing pyruvate kinase activity and reducing dihydroxyacetone (DHA) phosphorylation through decreased cellular ATP levels. It also exhibits potent antioxidant activity by curbing reactive oxygen species (ROS) production linked to DHA metabolism, although this antioxidant effect diminishes at higher concentrations (25–100 µM). Additionally, in alloxan-induced diabetic rat sciatic nerves, silibin prevented abnormal protein mono-ADP-ribosylation and maintained axonal transport of substance P-like immunoreactivity at micromolar doses (1 µM), suggesting its promise as a neuroprotective agent in diabetic neuropathy [72].

In Vivo Animal Studies: In alloxan-induced diabetic rats, silibin administration lowered serum glucose levels and protected pancreatic tissue from lipid peroxidation, evidenced by normalized malondialdehyde (MDA) and glutathione levels. Treatment with silymarin restored MDA levels to baseline within nine weeks and normalized insulin levels after one week. Furthermore, gene expression analyses using RT-PCR revealed normalization of insulin and Pdx1 mRNA—an important regulator of insulin promoter activity—along with recovery of pancreatic islet morphology in treated animals [73].

Human Studies

Clinical evidence supports silimarin's efficacy in improving glycemic control among diabetic patients, including those with alcoholic liver cirrhosis. A daily dose of 600 mg of silymarin over six months significantly reduced fasting and mean daily glucose levels, with improvements noted from the second month onward. This treatment also lowered insulin requirements by approximately 20%, indicating enhanced insulin sensitivity. Additionally, there was a modest reduction in HbA1c (0.5%) and oxidative stress markers such as MDA, alongside high patient satisfaction. In diabetic patients with end-stage renal disease, intravenous silibin (350 mg over 24 hours) restored thiol status in peripheral blood lymphocytes and

improved T-cell activation by reducing TNF-α mediated inflammation, potentially through modulation of γ-glutamyl transferase and enzymes critical for glutathione synthesis [74].

Safety Profile

Oral administration of silymarin at doses ranging from 140 to 700 mg every eight hours for seven days was well tolerated in non-cirrhotic patients with chronic hepatitis C. No significant adverse effects or laboratory abnormalities were reported; mild nausea and headache occurred but were deemed unrelated to silymarin [75].

Pharmacokinetics and Bioavailability

Silibin is rapidly absorbed in the stomach; however, its bioavailability is limited due to poor aqueous solubility and extensive first-pass metabolism. In rat studies, intestinal absorption of silibin at 20 mg/kg was approximately 35%, with peak plasma levels observed 30 minutes post-ingestion. Conjugation reactions in the liver (sulfation and glucuronidation) further reduce the circulating free silibin, with approximately 90% bound to conjugated metabolites. Parenteral administration or formulations incorporating mixed micelles with phosphatidylcholine and bile salts have demonstrated improved bioavailability and therapeutic efficacy [76].

ADVERSE EFFECTS

Human clinical studies involving milk thistle seed extract (silymarin) have consistently shown it to be safe and well tolerated, with minimal adverse effects reported. Typical adult dosages ranging from 200 to 900 mg per day, divided into two or three doses, generally produce no significant toxicity or side effects. Higher doses exceeding 1500 mg per day may occasionally cause mild gastrointestinal symptoms, such as a laxative effect attributed to increased bile secretion and flow.

Mild allergic reactions—including pruritus, urticaria, and arthralgia—have been observed but are infrequent and rarely necessitate discontinuation of therapy. Other reported adverse effects in clinical trials include bloating, dyspepsia, epigastric discomfort, flatulence, nausea, irregular stools, headaches, and dermatological symptoms; however, these occurred in only 2 to 10% of patients.

Animal toxicity studies further support the safety profile of silymarin. Oral administration of doses as high as 2500 to 5000 mg/kg body weight in rats and mice produced no observable toxic effects. Similar findings have been reported in rabbits and dogs, with no evidence of prenatal or postnatal toxicity. These data suggest that silymarin has a very low acute toxicity.

At higher concentrations, silymarin has demonstrated inhibitory effects on hepatic cytochrome P450 enzymes involved in drug metabolism (both phase I and phase II biotransformation systems). However, therapeutic plasma levels in humans are typically too low to cause clinically relevant drug interactions.

The safety of milk thistle during pregnancy and lactation has not been adequately studied in humans, though it is traditionally regarded as safe during breastfeeding. No clinical trials have been conducted in pediatric populations, and no specific contraindications have been identified to date [8].

EFFICACY ANALYSIS

The therapeutic benefit of silymarin in chronic viral hepatitis remains controversial. A systematic review assessing silymarin's efficacy specifically in chronic hepatitis B and C identified only a limited number of clinical trials: four studies involving hepatitis C patients, one involving hepatitis B patients, and two with unspecified chronic viral hepatitis populations. Notably, only one trial exclusively included hepatitis C patients, with the others either including mixed or unspecified cohorts.

Across these studies, silymarin administration was associated with reductions in serum transaminase levels compared to baseline values, though only one trial demonstrated a significant decrease relative to placebo. Importantly, no evidence supported silymarin's ability to reduce viral load or improve liver histology in either hepatitis B or C infections.

Overall, while silymarin may lower serum transaminases in patients with chronic viral hepatitis, it appears not to affect viral replication or histopathological liver damage. Consistent with this, a separate report concluded that silymarin does not influence elevated aminotransferase levels in chronic hepatitis patients [1,77].

CONCLUSION

Milk thistle (*Silybum marianum*) and its principal bioactive constituent, silymarin, continue to demonstrate considerable promise as therapeutic agents, particularly in the treatment of liver diseases and conditions associated with oxidative stress and inflammation. With a rich phytochemical profile dominated by flavonolignans—notably silybin (silibinin)—milk thistle exhibits a wide range of pharmacological activities, including hepatoprotective, antioxidant, anti-inflammatory, antifibrotic, and even anticancer effects. However, its limited bioavailability remains a major challenge, necessitating advanced drug delivery strategies. Although preclinical and early clinical data are encouraging, rigorous, large-scale randomized controlled trials are needed to confirm its efficacy and safety across diverse populations. This has led to the development of novel formulations including phytosomes, liposomes, nanoparticles, and complexes with bile salts, which have shown improved pharmacokinetic profiles and enhanced therapeutic efficacy in preliminary studies. Milk thistle stands as a compelling example of a traditional medicinal plant transitioning into evidence-based clinical practice. With its multifaceted therapeutic potential, particularly in liver health, silymarin represents a promising natural agent deserving of further scientific validation. Addressing current challenges through multidisciplinary research and clinical rigor will be key to unlocking its full pharmacological value in modern medicine.

REFERENCES

1. Das SK, Mukherjee S, Vasudevan DM. Medicinal properties of milk thistle with special reference to silymarin: An overview. NPR. 2008 Jan 1;7(2):182-92.

2. Varshney H, Yadav R. A Comprehensive Review On Milk Thistle: Phytochemistry, Pharmacokinetics, Mechanism Of Action, And Therapeutic Potentials.

3. DerMarderosian, A. The Reviews of natural products. 1st ed. Facts and Comparisons: St. Louis, Missouri,2001.

4. Evans WC. Trease and Evans' pharmacognosy. Elsevier Health Sciences; 2009 May 27.

5. Brandon-Warner E, Eheim AL, Foureau DM, Walling TL, Schrum LW, McKillop IH. Silibinin (Milk Thistle) potentiates ethanol-dependent hepatocellular carcinoma progression in male mice. Cancer letters. 2012 Dec 29;326(1):88-95.

6. Brantley SJ, Oberlies NH, Kroll DJ, Paine MF. Two flavonolignans from milk thistle (Silybum marianum) inhibit CYP2C9-mediated warfarin metabolism at clinically achievable concentrations. The Journal of pharmacology and experimental therapeutics. 2010 Mar 1;332(3):1081-7.

7. Chambers CS, Holečková V, Petrásková L, Biedermann D, Valentová K, Buchta M, Křen V. The silymarin composition… and why does it matter???. Food research international. 2017 Oct 1;100:339-53.

8. Bhattacharya S. Phytotherapeutic properties of milk thistle seeds: An overview. J Adv Pharm Educ Res. 2011;1:69-79.

9. DerMarderosian, A. The Reviews of natural products. 1st ed. Facts and Comparisons: St. Louis, Missouri, 2001.

10. Barceloux, D.G. Medical Toxicology of Natural Substances. John Wiley & Sons, Inc.:
New Jersey, 2008.

11. Pradhan SC and Girish C, Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine, Indian J Med Res, 2006, 124, 491-504.

12. Lee DY and Liu Y, Molecular Structure and Stereochemistry of Silybin A, Silybin B,
Isosilybin A and Isosilybin B, Isolated from Silybum marianum (Milk thistle), J Nat Prod, 2003, 66(9), 1171-1174.

13. Flora K, Hahn M, Rosen H and Benner K, Milk thistle (Silybum marianum) for the therapy of liver disease, Am J Gastroenterol, 1998, 93(2), 139-143.

14. Boigk G, Stroedter L, Herbst H, Waldschmidt J, Riecken EO and Schuppan D, Silymarin retards collagen accumulation in early and advanced biliary fibrosis secondary to complete bile duct obliteration in rats, Hepatology, 1997, 26(3), 643-649.

15. FizaAmjad, RidaZainab, Muhammad Akram, Umme Laila, MominaIftikhar, Adonis Sfera, PragneshParmar. A Review on Anti-tuberculosis Properties of Medicinal Plants. IAIM, 2023; 10(10): 1-9.

16.Muhammad Akram, Muhammad Amjad Chishti, Umme Laila, RidaZainab, MominaIftikhar, Muhammad Talha Khalil, AtheerKadhimIbadi, AymenHawani, Adonis Sfera, PragneshParmar. Herbal Remedies with potential COVID-19 Activities. IAIM, 2023; 10(9): 27-36.

17. Parmar P. Evolution of Toxicology in India: From Safety Concerns to Comprehensive Science. Advances in Clinical Toxicology. 2023;8(3):1-3.

18. Zhu HJ, Brinda BJ, Chavin KD, Bernstein HJ, Patrick KS, Markowitz JS. An assessment of pharmacokinetics and antioxidant activity of free silymarin flavonolignans in healthy volunteers: a dose escalation study. Drug Metabolism and Disposition. 2013 Sep 1;41(9):1679-85.

19. Zhang W, Hong R, Tian T. Silymarin's protective effects and possible mechanisms on alcoholic fatty liver for rats. Biomolecules & therapeutics. 2013 Jul 30;21(4):264.

20. Hoh C, Boocock D, Marczylo T, Singh R, Berry DP, Dennison AR, Hemingway D, Miller A, West K, Euden S, Garcea G. Pilot study of oral silibinin, a putative chemopreventive agent, in colorectal cancer patients: silibinin levels in plasma, colorectum, and liver and their pharmacodynamic consequences. Clinical Cancer Research. 2006 May 1;12(9):2944-50.

21. Sornsuvit C, Hongwiset D, Yotsawimonwat S, Toonkum M, Thongsawat S, Taesotikul W. The bioavailability and pharmacokinetics of silymarin SMEDDS formulation study in healthy Thai volunteers. Evidence-Based Complementary and Alternative Medicine. 2018;2018(1):1507834.

22. Xu Y, Li J, He B, Feng T, Liang L, Huang X. In vitro dissolution testing and pharmacokinetic studies of silymarin solid dispersion after oral administration to healthy pigs. Frontiers in Veterinary Science. 2022 Mar 1;9:815198.

23. Koltai T, Fliegel L. Role of silymarin in cancer treatment: facts, hypotheses, and questions. Journal of Evidence-Based Integrative Medicine. 2022 Jan;27:2515690X211068826.

24. Javed S, Kohli K, Ali M. Reassessing bioavailability of silymarin. Alternative medicine review. 2011 Sep 1;16(3):239.

25. Xie Y, Zhang D, Zhang J, Yuan J. Metabolism, transport and drug–drug interactions of silymarin. Molecules. 2019 Oct 14;24(20):3693.

26. Calani L, Brighenti F, Bruni R, Del Rio D. Absorption and metabolism of milk thistle flavanolignans in humans. Phytomedicine. 2012 Dec 15;20(1):40-6.

27. Wu JW, Lin LC, Tsai TH. Drug–drug interactions of silymarin on the perspective of pharmacokinetics. Journal of ethnopharmacology. 2009 Jan 21;121(2):185-93.

28. Nasri H. Silymarin and its properties; a nephrology viewpoint. Journal of Renal Endocrinology. 2015 Jul 4;1(1):e09-.

29. Aziz M, Saeed F, Ahmad N, Ahmad A, Afzaal M, Hussain S, Mohamed AA, Alamri MS, Anjum FM. RETRACTED: Biochemical profile of milk thistle (Silybum Marianum L.) with special reference to silymarin content. Food science & nutrition. 2021 Jan;9(1):244-50.

30. Zhao Y, Zhou Y, Gong T, Liu Z, Yang W, Xiong Y, Xiao D, Cifuentes A, Ibáñez E, Lu W. The clinical anti-inflammatory effects and underlying mechanisms of silymarin. IScience. 2024 Nov 15;27(11).

31. Liu Z, Sun M, Wang Y, Zhang L, Zhao H, Zhao M. Silymarin attenuated paraquat-induced cytotoxicity in macrophage by regulating Trx/TXNIP complex, inhibiting NLRP3 inflammasome activation and apoptosis. Toxicology in Vitro. 2018 Feb 1;46:265-72.

32. Zinatizadeh, M.R., Schock, B., Chalbatani, G.M., Zarandi, P.K., Jalali, S.A., and Miri, S.R. (2021). The Nuclear Factor Kappa B (NF-kB) signaling in cancer development and
immune diseases. Genes Dis. 8, 287–297.

33. Song, J., Pan, W., Sun, Y., Han, J., Shi, W., and Liao, W. (2017). Aspergillus fumigatus induced early inflammatory response in pulmonary microvascular endothelial cells: Role of p38 MAPK and inhibition by silibinin. Int. Immunopharmacol. 49, 195–202.

34. Mai, D., Yang, C., Xue, Y., Wang, Y., and Yan, C. (2017). Investigation on silymarin impact on lipopolysaccharide induced inflammation model based on arachidonic acid metabolism pathway. Chin. J. Chromatogr. 35, 578–586.
35. Cho K, Lee HG, Piao JY, Kim SJ, Na HK, Surh YJ. Protective effects of silibinin on Helicobacter pylori-induced gastritis: NF-κB and STAT3 as potential targets. Journal of Cancer Prevention. 2021 Jun 30;26(2):118.

36. Alsaggar M, Bdour S, Ababneh Q, El-Elimat T, Qinna N, Alzoubi KH. Silibinin attenuates adipose tissue inflammation and reverses obesity and its complications in diet-induced obesity model in mice. BMC Pharmacology and Toxicology. 2020 Jan 23;21(1):8.

37. Chen YH, Lin H, Wang Q, Hou JW, Mao ZJ, Li YG. Protective role of silibinin against myocardial ischemia/reperfusion injury-induced cardiac dysfunction. International journal of biological sciences. 2020 Apr 27;16(11):1972.

38. Shen HH, Alex R, Bellner L, Raffaele M, Licari M, Vanella L, Stec DE, Abraham NG. Milk thistle seed cold press oil attenuates markers of the metabolic syndrome in a mouse model of dietary-induced obesity. Journal of food biochemistry. 2020 Dec;44(12):e13522.

39. Faixová D, Ratvaj M, Maruščáková IC, Hrčková G, Karaffová V, Faixová Z, Mudroňová D. Silybin showed higher cytotoxic, antiproliferative, and anti-inflammatory activities in the CaCo cancer cell line while retaining viability and proliferation in normal intestinal IPEC-1 cells. Life. 2023 Feb 10;13(2):492.

40. Serçe A, Toptancı BÇ, Tanrıkut SE, Altaş S, Kızıl G, Kızıl S, Kızıl M. Assessment of the antioxidant activity of Silybum marianum seed extract and its protective effect against DNA oxidation, protein damage and lipid peroxidation. Food technology and biotechnology. 2016 Dec 2;54(4):455-61.

41. Asghar Z, Masood Z. Evaluation of antioxidant properties of silymarin and its potential to inhibit peroxyl radicals in vitro. Pakistan Journal of Pharmaceutical Sciences. 2008 Jul 1;21(3).

42. Fehér J, Láng I, Nékám K, Csomós G, Müzes G, Deák G. Effect of silibinin on the activity and expression of superoxide dismutase in lymphocytes from patients with chronic alcoholic liver disease. Free radical research communications. 1987 Jan 1;3(6):373-7.

43. Lettéron P, Labbe G, Degott C, Berson A, Fromenty B, Delaforge M, Larrey D, Pessayre D. Mechanism for the protective effects of silymarin against carbon tetrachloride-induced lipid peroxidation and hepatotoxicity in mice: evidence that silymarin acts both as an inhibitor of metabolic activation and as a chain-breaking antioxidant. Biochemical pharmacology. 1990 Jun 15;39(12):2027-34.

44. Anupam Sharma AS, Chakraborti KK, Handa SS. Antihepatotoxic activity of some Indian herbal formulations as compared to silymarin.

45. Muriel P, Mourelle M. The role of membrane composition in ATPase activities of cirrhotic rat liver: effect of silymarin. Journal of applied toxicology. 1990 Aug;10(4):281-4.

46. Favari L, Pérez Alvárez V. Comparative effects of colchicine and silymarin on CCI4-chronic liver damage in rats. Arch. Med. Res. 1997:11-7.

47. Reddy AC, Lokesh BR. Effect of curcumin and eugenol on iron-induced hepatic toxicity in rats. Toxicology. 1996 Jan 22;107(1):39-45.

48. Saratikov AS, Vengerovskiĭ AI, Sedykh IM. The correction with hepatic protectors of structural metabolic disorders in the liver in D-galactosamine poisoning. Farmakologiia i Toksikologiia. 1990 Mar 1;53(2):38-40.

49. Das SK, Vasudevan DM. Alcohol-induced oxidative stress. Life sciences. 2007 Jun 27;81(3):177-87.

50. Lieber CS. New concepts of the pathogenesis of alcoholic liver disease lead to novel treatments. Current gastroenterology reports. 2004 Jan;6(1):60-5.

51. Arteel G, Marsano L, Mendez C, Bentley F, McClain CJ. Advances in alcoholic liver disease. Best Practice & Research Clinical Gastroenterology. 2003 Aug 1;17(4):625-47.

52. Das SK, Vasudevan DM. Protective effects of silymarin, a milk thistle (Silybium marianum) derivative on ethanol-induced oxidative stress in liver. Indian Journal of Biochemistry and Biophysics. 2006 Oct 1;43(5):306.

53. Parés A, Planas R, Torres M, Caballería J, Viver JM, Acero D, Panés J, Rigau J, Santos J, Rodés J. Effects of silymarin in alcoholic patients with cirrhosis of the liver: results of a controlled, double-blind, randomized and multicenter trial. Journal of hepatology. 1998 Apr 1;28(4):615-21.

54. Bunout D, Hirsch S, Petermann M, De La Maza MP, Silva G, Kelly M, Ugarte G, Iturriaga H. Controlled study of the effect of silymarin on alcoholic liver disease. Revista Médica de Chile. 1992 Dec 1;120(12):1370-5.

55. Wang Y, Yuan AJ, Wu YJ, Wu LM, Zhang L. Silymarin in cancer therapy: Mechanisms of action, protective roles in chemotherapy-induced toxicity, and nanoformulations. Journal of Functional Foods. 2023 Jan 1;100:105384.

56. Singh RP, Dhanalakshmi S, Tyagi AK, Chan DC, Agarwal C, Agarwal R. Dietary feeding of silibinin inhibits advance human prostate carcinoma growth in athymic nude mice and increases plasma insulin-like growth factor-binding protein-3 levels. Cancer research. 2002 Jun 1;62(11):3063-9.

57. Lahiri-Chatterjee M, Katiyar SK, Mohan RR, Agarwal R. A flavonoid antioxidant, silymarin, affords exceptionally high protection against tumor promotion in the SENCAR mouse skin tumorigenesis model. Cancer Research. 1999 Feb 1;59(3):622-32.

58. Singh RP, Sharma G, Dhanalakshmi S, Agarwal C, Agarwal R. Suppression of advanced human prostate tumor growth in athymic mice by silibinin feeding is associated with reduced cell proliferation, increased apoptosis, and inhibition of angiogenesis. Cancer Epidemiology Biomarkers & Prevention. 2003 Sep 1;12(9):933-9.

59. Yanaida Y, Kohno H, Yoshida K, Hirose Y, Yamada Y, Mori H, Tanaka T. Dietary silymarin suppresses 4-nitroquinoline 1-oxide-induced tongue carcinogenesis in male F344 rats. Carcinogenesis. 2002 May 1;23(5):787-94.

60. Bhatia N, Zhao J, Wolf DM, Agarwal R. Inhibition of human carcinoma cell growth and DNA synthesis by silibinin, an active constituent of milk thistle: comparison with silymarin. Cancer letters. 1999 Dec 1;147(1-2):77-84.

61. Polyak SJ, Ferenci P, Pawlotsky JM. Hepatoprotective and antiviral functions of silymarin components in hepatitis C virus infection. Hepatology. 2013 Mar;57(3):1262-71.

62. Buzzelli G, Moscarella S, Giusti A, Duchini A, Marena C, Lampertico M. A pilot study on the liver protective effect of silybin-phosphatidylcholine complex (IdB1016) in chronic active hepatitis. International journal of clinical pharmacology, therapy, and toxicology. 1993 Sep 1;31(9):456-60.

63. Marcelli R, Bizzoni P, Conte D, Lisena MO, Lampertico M, Marena C, De Marco MF, Del Ninno E. Randomized controlled study of the efficacy and tolerability of a short course of IdB 1016 in the treatment of chronic persistent hepatitis. Eur Bull Drug Res. 1992;1(3):131-5.

64. Federico A, Trappoliere M, Tuccillo C, De Sio I, Di Leva A, Blanco CD, Loguercio C. A new silybin-vitamin E-phospholipid complex improves insulin resistance and liver damage in patients with non-alcoholic fatty liver disease: preliminary observations. Gut. 2006 Jun 1;55(6):901-2.

65. Loguercio C, Andreone P, Brisc C, Brisc MC, Bugianesi E, Chiaramonte M, Cursaro C, Danila M, de Sio I, Floreani A, Freni MA. Silybin combined with phosphatidylcholine and vitamin E in patients with nonalcoholic fatty liver disease: a randomized controlled trial. Free Radical Biology and Medicine. 2012 May 1;52(9):1658-65.

66. Fuchs EC, Weyhenmeyer R, Weiner OH. Effects of silibinin and of a synthetic analogue on isolated rat hepatic stellate cells and myofibroblasts. Arzneimittel-forschung. 1997 Dec 1;47(12):1383-7.

67. He Q, Osuchowski MF, Johnson VJ, Sharma RP. Physiological Responses to a Natural Antioxidant Flavonoid Mixture, Silymarin, in BALB/c Mice: I Induction of Transforming Growth Factor β1 and c-myc in Liver with Marginal Effects on Other Genes. Planta medica. 2002 Aug;68(08):676-9.

68. Kittur S, Wilasrusmee S, Pedersen WA, Mattson MP, Straube-West K, Wilasrusmee C, Jubelt B, Kittur DS. Neurotrophic and neuroprotective effects of milk thistle (Silybum marianum) on neurons in culture. Journal of Molecular Neuroscience. 2002 Jun;18(3):265-9.

69. Zi X, Zhang J, Agarwal R, Pollak M. Silibinin up-regulates insulin-like growth factor-binding protein 3 expression and inhibits proliferation of androgen-independent prostate cancer cells. Cancer research. 2000 Oct 15;60(20):5617-20.

70. Kazazis CE, Evangelopoulos AA, Kollas A, Vallianou NG. The therapeutic potential of milk thistle in diabetes. The review of diabetic studies: RDS. 2014 Aug 10;11(2):167.

71. Santos JO, Mira LB, Freire AM, Azevedo MA, Manso CA. Placental aldose reductase inhibition by Silybin (preliminary communication). Acta Médica Portuguesa. 1984;5(4-5):115-7.

72. Detaille D, Sanchez C, Sanz N, Lopez-Novoa JM, Leverve X, El-Mir MY. Interrelation between the inhibition of glycolytic flux by silibinin and the lowering of mitochondrial ROS production in perifused rat hepatocytes. Life sciences. 2008 May 23;82(21-22):1070-6.

73. Soto C, Mena R, Luna J, Cerbon M, Larrieta E, Vital P, Uria E, Sanchez M, Recoba R, Barron H, Favari L. Silymarin induces recovery of pancreatic function after alloxan damage in rats. Life Sciences. 2004 Sep 17;75(18):2167-80.

74. Dietzmann J, Thiel U, Ansorge S, Neumann KH, Tüger M. Thiol-inducing and immunoregulatory effects of flavonoids in peripheral blood mononuclear cells from patientswith end-stage diabetic nephropathy. Free Radic Biol Med 2002. 33(10):1347-1354.

75. Hawke RL, Schrieber SJ, Soule TA, Wen Z, Smith PC, Reddy KR, Wahed AS, Belle SH, Afdhal NH, Navarro VJ, Berman J. Silymarin ascending multiple oral dosing phase I study in noncirrhotic patients with chronic hepatitis C. The Journal of Clinical Pharmacology. 2010 Apr;50(4):434-49.

76. Tamayo C, Diamond S. Review of clinical trials evaluating safety and efficacy of milk thistle (Silybum marianum [L.] Gaertn.). Integrative cancer therapies. 2007 Jun;6(2):146-57.

77. Mayer KE, Myers RP, Lee SS. Silymarin treatment of viral hepatitis: a systematic review. Journal of viral hepatitis. 2005 Nov;12(6):559-67.