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Review

Silymarin as a Redox-Signalling and Proteostasis Modulator

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Abstract

Silymarin (*Silybum marianum* (L.) Gaertn. extract) is a widely used botanical for liver disease, yet clinical results remain inconsistent. Most mechanistic work uses supraphysiological aglycones, whereas humans are exposed predominantly to phase II conjugates that are strongly protein-bound and routed by transporters toward bile and the intestinal mucosa. We reframe silymarin activity through a spatial pharmacology lens, proposing three post-intake windows: early (0–2 h) conjugate-dominant exposure with localised β-glucuronidase-mediated reactivation; intermediate (2–8 h) enterohepatic recirculation pulses; and late (8–48 h) microbial catabolite contributions. Each window engages distinct signalling modules—Keap1/NRF2, NF-κB, and AMPK-mTOR-TFEB—via transient redox events (quinone cycling, micro-H_2O_2 relays) and proteostatic remodelling (autophagy/mitophagy). We synthesise human pharmacokinetic and clinical evidence—with emphasis on MASLD and alcohol-associated liver disease—and show how formulation, meal timing, and microbiome metabotype determine which windows are engaged. Finally, we propose minimum reporting standards and falsifiable hypotheses to reduce between-study heterogeneity and enable precision use of silymarin.

Keywords: silymarin; silybin; flavonolignans; enterohepatic recirculation; transporter topology; phase II conjugation; redox signalling; NRF2; proteostasis

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1. Introduction

Silymarin, a flavonolignan-rich extract from *Silybum marianum* (L.) Gaertn., is among the most widely used botanicals for liver support in contemporary practice. People use it for metabolic-associated steatotic liver disease, alcohol-associated liver disease, and other inflammatory liver conditions, often alongside dietary changes, exercise programmes, and standard pharmacotherapy. Despite decades of publications, translation remains uneven, because trials differ in product quality, dosing context, adherence patterns, and the endpoints chosen to define meaningful clinical improvement across countries, clinical settings, and diverse patient populations today [1], Figure 1.

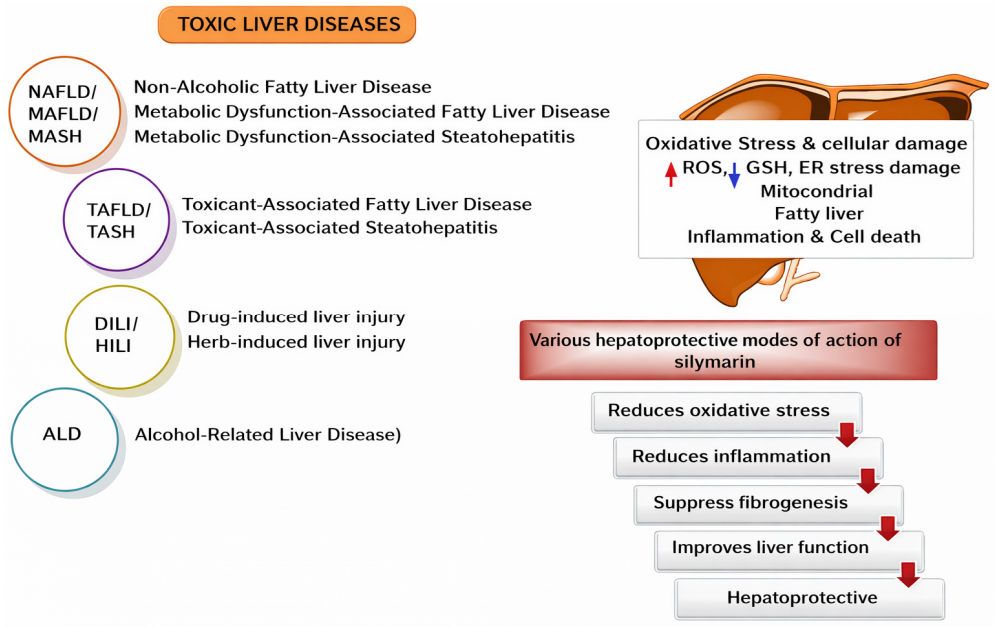

Figure 1. The common mechanism of oxidative stress underlies hepatocellular damage. A common strategy for treating toxic liver disease (TLD) is to use antioxidants as hepatoprotective agents. Available preclinical and clinical evidence indicates that silymarin is a hepatoprotective agent with established antioxidant, anti-inflammatory, and antifibrotic effects. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated by mitochondrial dysfunction, cytochrome P450 activity, and immune cell activation trigger lipid peroxidation, protein carbonylation, and DNA oxidation, collectively impairing hepatocellular integrity. Silymarin flavonolignans—principally silybin A/B, isosilybin, silychristin, and silydianin—counteract this cascade through at least three complementary mechanisms: (i) direct radical scavenging and iron chelation that interrupt lipid peroxidation chains; (ii) electrophile-mediated activation of the Keap1/NRF2/ARE axis, upregulating endogenous antioxidant enzymes (NQO1, HO-1, GCLC) and restoring glutathione reserves; and (iii) context-dependent modulation of NF-κB inflammatory signalling, reducing cytokine-driven amplification of oxidative injury without suppressing innate immune competence. The net result is a shift from pro-oxidant to cytoprotective tone at the gut–liver interface, forming the mechanistic basis for the hepatoprotective, anti-inflammatory, and antifibrotic effects documented in clinical and preclinical studies.

A persistent problem is that many mechanistic narratives are derived from continuous exposure to unconjugated aglycones at concentrations that humans rarely reach in vivo. After oral intake, flavonolignans are rapidly glucuronidated and sulphated in enterocytes and hepatocytes, and most circulating material is strongly protein-bound, which keeps the free aglycone low. Transporter-driven routing enriches bile and the intestinal mucosa, and enterohepatic cycling can create secondary exposure waves hours after. When these compartments and time patterns are ignored, low plasma aglycone is easily misread as a weak biological opportunity [2].

The growing scientific interest in silymarin is reflected in bibliometric trends. A search of PubMed and Web of Science using the terms “silymarin” or “silybin” retrieves more than 5000 indexed records as of 2024, with annual publication counts rising from approximately 100 articles per year in the early 2000s to over 400 per year in the 2020–2024 period. Research activity has increasingly shifted toward pharmacokinetics, formulation science, and molecular signalling, mirroring the mechanistic reorientation proposed in this review. Notably, clinical trial registrations have also grown, yet the proportion of studies reporting exposure-verified metabolite data remains low, underscoring the standardisation gap that this review aims to address.

This review, therefore, reframes silymarin through a spatial pharmacology lens, emphasising place, timing, and chemical speciation as core determinants of effect. In inflamed microenvironments, where oxidases, metals, and disrupted thiol buffering coexist, local deconjugation can regenerate brief aglycone and quinone pulses from phase II pools. These short events may tune Keap1/NRF2 thresholds, temper context-dependent NF-κB activation, and initiate proteostasis programmes that outlast the initiating chemistry.

Our goal is to align mechanistic claims with human-relevant exposure by integrating pharmacokinetics, transporter topology, and signalling modules that remain plausible at realistic, unbound concentrations. We use a time window framework, from 0 to 2 h, from 2 to 8 h, and from 8 to 48 h, to connect exposure pulses with downstream remodelling, including AMPK mTOR TFEB coupling, autophagy flux, and mitochondrial quality control. We also discuss why formulation, meal timing, circadian rhythms, and microbiome metabotypes can redirect exposure and produce responder subgroups in heterogeneous trials. Finally, we propose minimum reporting standards and testable hypotheses, so future studies can be compared and interpreted with greater confidence.

2. Scope and Structure of the Review

This review examines silymarin as a spatially gated signalling mixture, rather than as a generic antioxidant supplement. We focus on human-relevant exposure, emphasising isomer composition, phase II conjugation, protein binding, and transporter-driven routing that enriches bile and the intestinal mucosa. We then integrate these pharmacokinetic features with local reactivation mechanisms, including beta-glucuronidase and sulphatase hotspots, quinone cycling, and micro-H_2O_2 relays that shape redox microenvironments.

To connect chemistry with biology, we use a three-window framework, early, intermediate, and late, and we map each window to transcription factor modules, NRF2 and NF-kB, metabolic proteostatic coupling through AMPK, mTOR, TFEB, and downstream outcomes in autophagy, mitophagy, and broader proteostasis. Finally, we synthesise clinical evidence, with emphasis on MASLD and alcohol-associated liver disease, and we explain sources of heterogeneity related to formulation, timing, metabotype, and endpoints. The review concludes with minimum reporting standards and falsifiable hypotheses designed to improve interpretability, reproducibility, and precision use in future trials.

3. Silymarin Chemical Space and Bioactive Species

Silymarin is best treated as a defined chemical mixture, not as a single active ingredient, because its biological interpretation depends on which flavonolignans and companion phenolics are actually present. The extract typically contains silybin isomers, isosilybins, silychristin, and silydianin, with additional constituents that vary with cultivation and extraction conditions. This compositional variability matters because different members of the mixture differ in polarity, protein binding, conjugation patterns, and transporter handling, which together shape where exposure occurs in the gut–liver axis. For that reason, any mechanistic or clinical discussion must begin with clear composition and standardisation, including batch fingerprints and transparent analytical methods, before moving to pharmacokinetics and signalling [3], Figure 2.

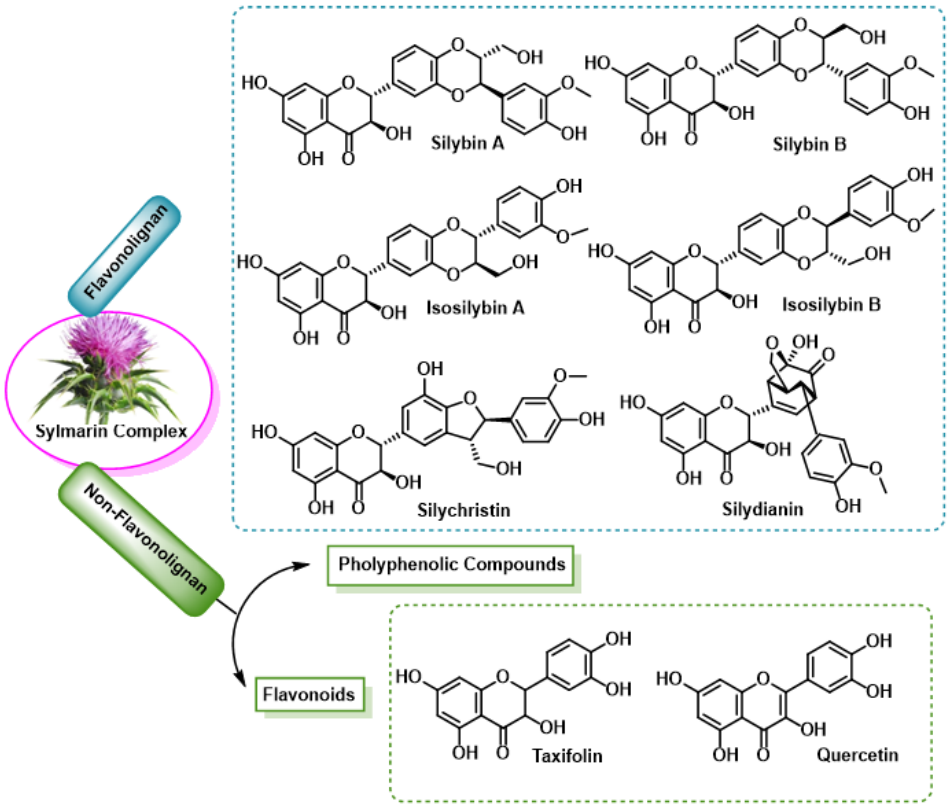

Figure 2. Different components of silymarin with their molecular structures.

3.1. What "Silymarin" Is in Practice: Composition and Standardisation

In practice, silymarin is not a single molecule; it is a botanical extract obtained mainly from the fruits of *Silybum marianum* (L.) Gaertn., often called milk thistle. The term is frequently used loosely in papers and product labels, sometimes referring to the whole extract and other times to isolated silybin, which creates confusion from the very first sentence of a study. A useful starting point is to treat silymarin as a defined chemical mixture, whose biological interpretation depends on what is actually present in that mixture, and in what relative proportions [4].

Chemically, most preparations contain a family of flavonolignans, typically including silybin and its isomers, isosilybin, silychristin, and silydianin, with smaller contributions from related phenolics such as taxifolin. The relative abundance of these constituents varies with plant genetics, growing conditions, harvest timing, storage, and extraction parameters such as solvent composition, temperature, and purification steps. These differences are not cosmetic, because isomers can differ in polarity, protein binding, metabolism, and transporter interactions, and therefore can yield distinct exposure patterns even when the same nominal dose is administered [5], Figure 3.

Standardisation usually relies on quantifying a set of marker compounds by chromatographic methods, most commonly HPLC with UV detection, increasingly complemented by LC-MS for improved selectivity. While "total silymarin" is often reported as a single percentage, this aggregated number can hide meaningful shifts in the underlying profile, especially when one constituent is enriched at the expense of others. Reliable standardisation should therefore include batch-specific fingerprints, isomer-aware quantification where feasible, and clear reference standards, since different calibrants can produce different apparent totals for the same sample [6], Figure 4.

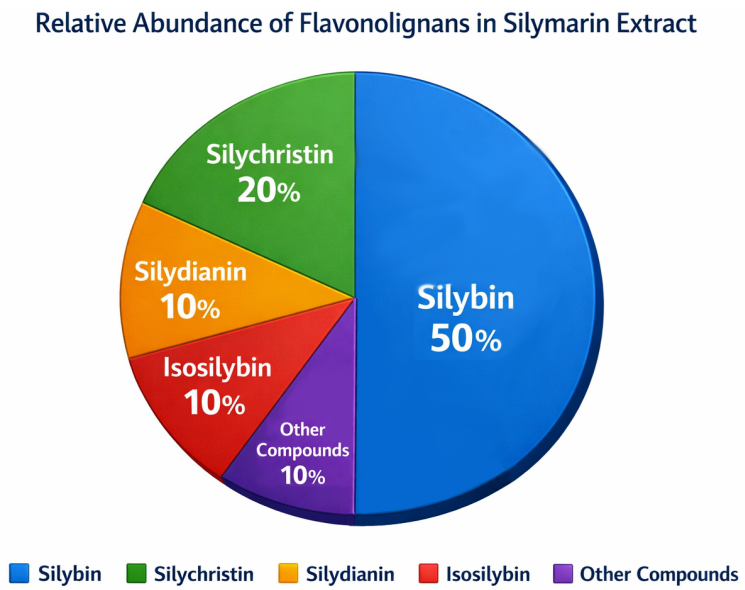

Figure 3. The major and minor flavonolignans present in silymarin extract are summarised, including their relative abundance. Silybin (silybinin) is the dominant active component, accounting for approximately 40% to 60% of total silymarin and acting as the main hepatoprotective and antioxidant agent. The approximate composition of a typical standardised extract is: silybin A + B ~50%, isosilybin A + B ~10%, silychristin ~20%, silydianin ~10%, and taxifolin and other phenolics ~10%. These proportions vary with cultivar, extraction solvent, and purification method. A bar or pie chart representation of these values would more intuitively convey the dominance of silybin and the minor-component nature of the remaining flavonolignans; the present structural illustration complements that quantitative perspective.

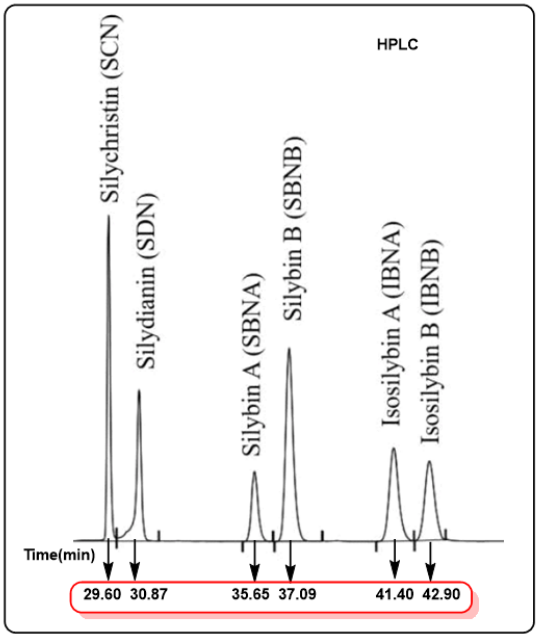

Figure 4. HPLC retention times of the six flavonolignans in silymarin [7].

For a review that aims to be clinically credible, it helps to insist on transparent reporting, including extract source, drug-to-extract ratio, solvent system, excipients, and stability conditions, alongside an analyte list with quantitative ranges. When authors present these details consistently, studies become comparable across laboratories, and mechanistic claims can be tied to the actual chemical entities that patients and participants are exposed to.

3.2. Major Flavonolignans and Isomerism (e.g., Silybin A/B) and Why It Matters

Silymarin derives much of its complexity from a small family of flavonolignans, with silybin as the best-known representative. What many studies call "silybin" is usually a mixture of two diastereoisomers, silybin A and silybin B, and a similar pairing exists for isosilybin A and isosilybin B. These molecules share the same formula but differ in three-dimensional arrangement, which can alter binding to enzymes, transporters, and even serum proteins [8], Figure 5.

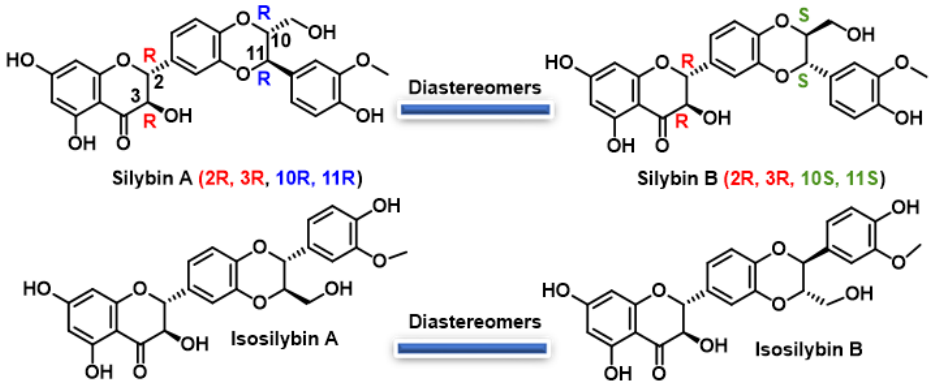

Figure 5. Structures and chirality of silybin and isosilybin diastereomers.

Most extracts also contain silychristin and silydianin, with smaller contributions from related phenolics such as taxifolin. Each constituent presents a distinct pattern of hydroxyl groups and steric constraints, so conjugation by UGT and SULT enzymes does not proceed uniformly across the mixture. As a result, the dominant glucuronides and sulphates can differ between batches, and between individuals, even when the label claim is identical [9].

Isomerism matters biologically because many experiments and clinical discussions implicitly assume that all "silybin" behaves as one entity. In reality, the A and B isomers can differ in polarity, protein binding, and transporter preference, which affects their routing into bile and their persistence in intestinal and hepatic microenvironments. Those exposure differences can translate into different thresholds for redox and electrophile signalling, and into distinct patterns of transcriptional modulation [10].

Analytically, these distinctions are easy to miss because coelution is common with routine HPLC UV assays that report a single peak area. More selective LC-MS workflows and, where feasible, chiral separations can quantify the major isomers and their conjugates with far greater confidence. For a review aimed at clinical relevance, reporting isomer-resolved composition should be treated as a basic quality marker, not an optional refinement [11].

3.3. Physicochemical Determinants: Solubility, Micellarization, Protein Binding, and Free Fraction

The physicochemical profile of silymarin explains why its biology often looks inconsistent across models, even before metabolism is considered. Most flavonolignans are poorly water-soluble, and their dissolution in gastrointestinal fluids can become the rate-limiting step for absorption. This matters because a nominal oral dose may translate into very different luminal concentrations, depending on particle size, excipients, and the presence of dietary lipids [12].

Once in the intestine, micellarization is a second decisive bottleneck because bile salts and phospholipids can solubilize otherwise insoluble flavonolignans into mixed micelles. In practice, this means that food, bile flow, and intestinal motility can reshape exposure, not by changing the intrinsic chemistry, but by changing how much of the mixture becomes available at the epithelial surface. It also means that in vitro work performed in simple

aqueous buffers may systematically underestimate uptake, unless the model reproduces relevant colloidal environments [13].

After absorption, protein binding becomes central to interpretation, because silymarin constituents and especially their conjugates can bind strongly to albumin and other plasma proteins. Total plasma levels, therefore, provide only limited insight into the concentrations that are free to diffuse, enter cells, or engage transporters at membranes. The unbound fraction is usually the better proxy for pharmacological opportunity, yet it is rarely measured directly, and it can shift with inflammation, fatty acids, and co-administered drugs [14].

For a mechanistically credible narrative, it helps to separate what increases total exposure from what increases unbound exposure, because these are not always aligned. Studies should report formulation details, solubility conditions, and ideally unbound measurements obtained by equilibrium dialysis or ultra-filtration under controlled protein concentrations. When these determinants are made explicit, low plasma aglycone levels stop being a paradox and instead become an expected feature of a compound class whose effective biology is often spatially concentrated [15].

3.4. Human-Relevant Exposure: Phase II Conjugates as Dominant Circulating Species

In humans, the dominant systemic forms of silymarin are rarely the parent aglycones that dominate many cell culture experiments. After oral intake, silymarin flavonolignans undergo extensive first-pass metabolism, and glucuronidation and sulphation quickly convert them into more polar conjugates that circulate in plasma. As a consequence, measured blood concentrations often reflect a mixture of phase II metabolites, rather than free silybin or other unconjugated constituents [16].

This matters because conjugation changes not only clearance, but also where exposure is concentrated. Conjugates tend to be efficient substrates for transporter-driven routing, which can favour biliary secretion and repeated intestinal delivery, while keeping free aglycone levels in systemic circulation very low. In practical terms, a low plasma aglycone signal should not be interpreted as biological irrelevance, because it may coexist with high canalicular, luminal, or mucosal reservoirs that are simply not captured by routine sampling [17].

A metabolite-first perspective also improves how mechanistic claims are evaluated. Conjugates are not necessarily inert, since they can participate in local cycling through deconjugation in specific microenvironments, and they can shape exposure timing through enterohepatic recirculation. Secondary peaks and prolonged low-amplitude signals are therefore expected, especially when bile flow, microbiome activity, and meal timing vary between individuals [18].

For this reason, human-relevant studies benefit from profiling conjugate panels, not only parent compounds, and from reporting sampling times with care. When possible, unbound measurements and time-resolved metabolite ratios provide a clearer bridge between chemistry, spatial exposure, and plausible signalling effects [19].

3.5. Microbial Transformation and Metabotypes: What Is Known, What Needs Measuring

Microbial transformation is a plausible, and still undermeasured, driver of variability in silymarin responses. Although the parent flavonolignans are extensively conjugated by the host, repeated delivery of conjugates to the gut through bile creates opportunities for microbiome-dependent processing. In this setting, microbial enzymes can reshape both exposure duration and the chemical identities that ultimately contact the intestinal mucosa [20].

The best supported mechanism is deconjugation, since many gut communities express β glucuronidase and sulphatase activities that can regenerate aglycones locally. This does

not imply a sustained rise in systemic aglycone levels, rather it suggests brief, spatially restricted increases near epithelial and immune cells. Those short-lived pulses may be particularly relevant in inflamed microenvironments, where oxidative tone and metal availability can amplify signalling consequences [21].

Beyond deconjugation, more extensive microbial catabolism is likely, producing smaller phenolic fragments that may have their own transport, distribution, and receptor profiles. However, the field still lacks consistent metabolite maps in humans, especially in late time windows when microbial processing should be most visible. Without that information, it remains difficult to separate true pharmacology from dietary background noise [22].

What needs measuring is straightforward but rarely done: time-resolved panels of conjugates, aglycones, and downstream catabolites, paired with microbiome features that can explain them. Stool and urine metabolomics, targeted assays for key enzyme activities, and careful sampling at 6, 12, 24, and 48 h would allow metabotypes to be defined with confidence. Once those metabotypes are established, clinical trials can stratify participants more rationally, and mechanistic claims can be tied to measurable exposure patterns [23].

4. Spatial Pharmacokinetics of Silymarin: Routing, Barriers, and Enterohepatic Cycling

Spatial pharmacokinetics frames silymarin as a mixture whose effects depend on where metabolites travel, not only on how high plasma peaks appear. After oral intake, dissolution and micellar solubilization govern how much reaches the intestinal surface, and first-pass metabolism begins immediately. Enterocytes conjugate flavonolignans and route a portion back to the lumen, while the remainder enters portal blood and reaches hepatocytes, where conjugation and transporter-mediated secretion into bile intensify the bile-facing reservoir. This directional handling creates compartmentalised exposure, with repeated delivery to the gut–liver interface through enterohepatic cycling, and it explains why systemic aglycone levels can remain low while local signalling opportunity persists, [24]. As illustrated in Figure 6, the sequential steps of dissolution, enterocyte uptake, first-pass conjugation, biliary secretion, and enterohepatic recirculation define a spatial pharmacokinetic circuit rather than a simple absorption–distribution–metabolism–excretion sequence; understanding this circuit is essential for interpreting both the apparent low bioavailability of silymarin and its sustained biological activity at gut–liver axis compartments.

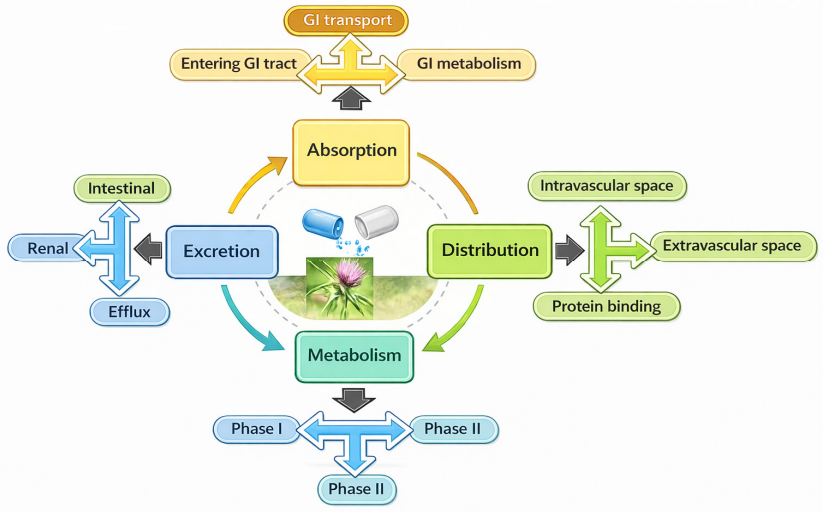

Figure 6. Pharmacokinetics of silymarin, showing the process by which silymarin is absorbed, distributed, metabolised and excreted from the body.

4.1. Absorption and First-Pass Metabolism

Oral absorption of silymarin is governed less by membrane permeability than by dissolution and solubilization in intestinal fluids. The flavonolignans are poorly soluble in water, so bile salts, dietary lipids, and formulation excipients largely determine how much material reaches the epithelial surface in an absorbable form [25].

At the enterocyte, uptake occurs in a crowded chemical environment that favours rapid metabolism. Conjugation by UDP glucuronosyltransferases and sulphotransferases can begin within the intestinal wall, and a portion of newly formed conjugates is immediately routed back into the lumen by apical efflux transporters. The same cells may also export conjugates basolaterally into portal blood, creating an early systemic signal that is metabolite-rich rather than aglycone-rich [26].

Portal delivery brings these species to hepatocytes, where first-pass metabolism further amplifies conjugation and reshapes the profile. Hepatic enzymes generate multiple glucuronides and sulphates, and canalicular transporters preferentially secrete many of them into bile, which establishes the characteristic bile-facing reservoir. Only a small fraction of parent aglycones typically escapes into the general circulation, and even that fraction is often protein-bound [27].

This two-stage filtering, enterocyte then hepatocyte, explains why plasma measurements alone can underestimate relevant exposure. Interindividual differences in conjugating capacity can be substantial during inflammation, ageing, and comedication. It also clarifies why meal timing, bile flow, and transporter variation can shift both peak timing and the balance between luminal and systemic compartments [28].

4.2. Conjugation Patterns + "Total vs. Unbound" Exposure

Silymarin exposure in humans is shaped by conjugation patterns that are both rapid and heterogeneous, because different flavonolignans present different hydroxyl topologies to UGT and SULT enzymes. The resulting plasma signal is, therefore, a moving mixture of glucuronides and sulphates, rather than a stable representation of one parent compound [29].

These conjugates differ in polarity and transporter affinity, so their ratios can hint at which tissues are being served at a given moment, especially along the gut–liver axis. Importantly, the same total concentration can correspond to very different biological opportunity, since most species are extensively protein-bound. Inflammation, hypoalbuminemia, and competing ligands can all increase free fractions, without changing totals, and alter response markedly clinically [30].

For this reason, total plasma levels are a blunt instrument, useful for confirming intake but poor at predicting cellular engagement. Unbound exposure, measured as the free fraction under physiologic protein concentrations, better reflects what can cross membranes, access intracellular redox microenvironments, or be handled by uptake transporters [31].

Practically, studies should report both totals and free fractions, and they should state the method used, such as equilibrium dialysis or validated ultrafiltration, together with recovery controls. When unbound data are paired with time-resolved metabolite profiling, apparent discrepancies between high doses and modest effects often become understandable [32].

4.3. Transporter Topology and "Bile-First" Logic

Transporter topology is central to understanding why silymarin behaves like a bile-first botanical, even when plasma levels appear modest. After absorption, flavonolignans and especially their conjugates meet a series of directional pumps that favour secretion, not simple diffusion. This architecture creates compartmentalised exposure, with repeated

delivery to bile and the intestinal lumen. It also means that low systemic aglycone does not rule out strong local signalling [33].

In enterocytes, apical efflux transporters can return conjugates to the lumen, limiting net portal entry while enriching the mucosal surface. Basolateral exporters feed the portal vein, yet the balance between the two routes depends on transporter expression, saturation, and the presence of bile salt micelles. These details help explain why formulation and meal timing can shift apparent bioavailability without changing intrinsic metabolism [34].

In hepatocytes, canalicular transporters drive the bile-first logic much more decisively. Many glucuronides and sulphates are preferentially secreted into bile, which concentrates them near cholangiocytes, the canalicular membrane, and downstream intestinal segments. Uptake transporters at the sinusoidal side can retrieve circulating species, tightening the loop and supporting secondary waves through entero-hepatic recirculation [35].

This vectorial routing implies that the relevant question is often where the molecules go, rather than how high the plasma peak rises. Transporter inhibition, induction, or genetic variation can therefore change clinical outcomes by redirecting exposure between portal blood, bile, and lumen. A transport informed design should measure metabolite profiles on both sides of polarised barriers whenever feasible [36].

4.4. Enterohepatic Recirculation, Mucosal Reservoirs, and Secondary Portal Waves

Enterohepatic recirculation is a defining feature of silymarin exposure, because biliary secretion repeatedly returns conjugates to the intestinal lumen. This cycling can extend apparent half-life, and it can generate secondary concentration waves that are invisible in sparse sampling designs. Interindividual differences in bile flow, transporter expression, and meal patterns can therefore amplify variability across trials [37].

Once conjugates reach bile, they encounter a mucosal landscape where diffusion is constrained but residence time can be long, especially within the unstirred layer and mucus. Mixed micelles formed by bile acids can keep flavonolignan species solubilized, raising local concentrations at the brush border without raising systemic free levels. The intestinal epithelium, therefore, experiences sustained contact with metabolite-rich mixtures, even when plasma profiles show only modest peaks [38].

Secondary portal waves arise when luminal conjugates are reabsorbed, either directly or after partial deconjugation, then delivered again to the liver through the portal vein. Reabsorption can occur in the small intestine and, for some species, later in the colon, which helps explain delayed peaks and long tails. These later signals often occur hours after intake, and they can coincide with postprandial bile flow or with changes in motility [39]. The role of microbial β-glucuronidase and sulphatase enzymes in generating these secondary aglycone pulses has been described in detail in Section 3.5; here the focus is on the pharmacokinetic consequences of reabsorption rather than on the microbial mechanisms themselves.

Mucosal reservoirs also imply that local immune and epithelial cells may integrate repeated low-amplitude exposures into durable transcriptional and proteostatic responses. For clinical translation, sampling should include late time points, and faecal or urinary metabolite panels [40].

5. Local Reactivation and Redox Microenvironments

Local reactivation explains how silymarin can generate biologically relevant signals even when circulating aglycones remain scarce. Most human exposure consists of glucuronides and sulphates, and these conjugates behave like a stored, transportable pool that can be converted back into a more reactive species only in the right place. At barrier surfaces and within bile-exposed niches, enzymatic activity and constrained diffusion allow

short-lived increases in aglycone availability near cells that decode redox and electrophile cues [41].

This section focuses on the microenvironments where deconjugation is most plausible and on why inflammation often amplifies the process. Rather than assuming uniform distribution, we consider how mucus, immune cell recruitment, and microbial ecology create hotspots that concentrate chemistry, and therefore determine which signalling programmes are actually engaged in vivo. Figure 7 depicts the three principal reactivation niches—the intestinal mucosa, the bile canalicular space, and the inflamed lamina propria—and shows how each is distinguished by enzyme availability, diffusion constraints, and redox tone, all of which together determine whether a conjugate pool becomes a signalling-competent aglycone microburst or remains pharmacologically inert.

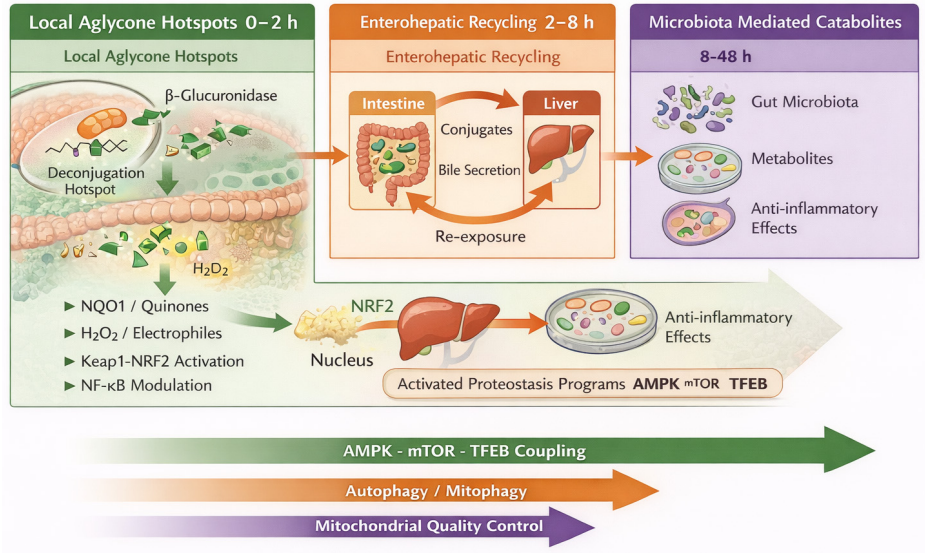

Figure 7. Local reactivation microenvironments along the gut–liver axis. Phase II conjugates are delivered to bile, and the intestinal surface can be locally deconjugated in enzyme-rich niches, generating short aglycone microbursts that feed quinone cycling and micro-H_2O_2 relays. Constrained diffusion and inflammation amplify these events near epithelial and immune targets, shaping downstream redox and electrophile signalling thresholds.

5.1. β-Glucuronidase/Sulphatase Hotspots

Phase -II conjugates dominate human silymarin exposure, yet they are not chemically terminal in every tissue. Beta-glucuronidase and sulphatase activities can regenerate aglycones locally, creating brief increases in reactive potential near cellular targets [42]. The microbial ecology and metabotype framework underpinning this deconjugation capacity was introduced in Section 3.5; the present section focuses specifically on where these enzymatic hotspots are located, how inflammation amplifies them, and what their redox consequences are for adjacent cells.

Hotspots occur along the intestinal mucosa, where bacterial enzymes act on biliary delivered conjugates within mucus and the unstirred layer. This process is uneven across the gut, reflecting regional microbiome composition, substrate availability, pH, and bile acid patterns that change after meals [43].

Inflammation amplifies these hotspots by recruiting immune cells that release lysosomal hydrolases, and by altering epithelial permeability and mucus structure. Inflamed niches also provide oxidants, transition metals, and disturbed thiol buffering, so small amounts of regenerated aglycone can trigger outsized signalling [44].

The practical implication is that plasma profiles can underestimate biologically relevant exposure at the gut–liver interface, where reactivation is most likely. Studies should

therefore quantify enzyme activity surrogates, such as faecal beta-glucuronidase, microbial gene abundance, or inhibitor sensitivity, alongside time-resolved metabolite panels in stool and urine. In mechanistic assays, co-culture models, defined enzyme additions, and selective inhibitors can separate conjugate driven effects from reactivation-driven effects, and clarify whether luminal, mucosal, or canalicular exposure dominates over time [45].

When trials report these variables, null results become interpretable, and positive signals can be linked to specific metabotypes and inflammatory contexts with confidence [46].

5.2. "Microbursts": Transient Aglycone Regeneration near Target Cells

The term microburst captures a simple idea: local chemistry can be intense even when systemic concentrations appear modest. After biliary delivery, silymarin conjugates can accumulate near epithelial surfaces, and enzymatic deconjugation may regenerate aglycones for minutes rather than hours. These brief events are hard to see in plasma, yet they can still shift signalling thresholds in cells that sit nearby [47]. It is important to acknowledge that, at present, no study has directly measured microburst events in human tissues in vivo. The microburst concept is supported by indirect evidence: (i) ex vivo demonstration of β-glucuronidase activity in human intestinal mucosa and inflammatory infiltrates; (ii) detection of unconjugated aglycones in bile and faecal samples at concentrations exceeding plasma levels; and (iii) pharmacokinetic modelling showing that local deconjugation rates can transiently exceed reconjugation capacity in inflamed niches. Direct validation would require spatial metabolomics or real-time redox imaging in mucosal biopsies collected at defined post-dose intervals, an approach that is technically feasible but has not yet been applied to silymarin. This remains an important gap that future studies should address.

Microbursts are most plausible at interfaces where conjugates linger, within mucus, within inflamed lamina propria, and near canalicular membranes. In those compartments, beta-glucuronidase and sulphatase activity can rise sharply, and diffusion is constrained by macromolecular crowding and limited convection. The regenerated aglycone can partition into membranes, encounter oxidases and redox active metals, and transiently form electrophilic intermediates before it is reconjugated or cleared [48].

Biologically, what matters is timing relative to cellular sensors that decode short pulses. Keap1 cysteines, NF-kB regulators, and stress kinases integrate transient redox and electrophile signals, and repeated pulses can approximate longer exposure when meals and bile flow recur. This framing helps reconcile why aglycone dosing in vitro often exaggerates effects, because continuous exposure bypasses the in vivo gating imposed by transport and metabolism [49].

Testing microbursts requires methods that respect compartment and time, not only concentration. Polarised co-cultures, rapid sampling, and targeted assays for both aglycones and conjugates should be paired with immediate transcriptional and proteostatic readouts. Where feasible, imaging of redox reporters in situ can localise the pulse and clarify its audience [50].

5.3. Quinone Cycling and Two-Electron Rescue (NQO1), Glutathione Economy

Quinone chemistry bridges silymarin exposure and redox signalling, because flavonolignans can access reversible oxidation states in living tissues. In oxidative microenvironments, catechol motifs can convert into quinones or semiquinones, especially near metal sites and active oxidases. These intermediates can cycle, generating localised reactive oxygen species that behave as short signals rather than diffuse toxins [51].

A key determinant of adaptive versus harmful chemistry is two-electron rescue capacity, prominently NAD(P)H:quinone oxidoreductase 1, known as NQO1. NQO1 reduces quinones directly to hydroquinones, limiting one-electron steps that propagate radical for-

mation and collateral damage. Because NQO1 is NRF2-regulated, early electrophile pulses can prime rescue on subsequent exposures. When NQO1 capacity is sufficient, electrophile tone can rise, yet it is buffered into reversible modifications that support NRF2-dependent transcription [52].

Glutathione economy complements NQO1, because conjugation and thiol exchange can neutralise intermediates and repair protein thiols. Glutathione is finite, and depletion can convert signalling pulses into stress that amplifies inflammation and mitochondrial dysfunction. This balance may differ between healthy liver, steatotic liver, and inflamed mucosa, where baseline redox state is shifted. Cysteine supply and GCL activity influence glutathione renewal and should be monitored clinically [53].

For interpretation, these principles suggest measuring quinone prone metabolites, NQO1 induction, and glutathione redox ratios over time. Short lived changes in protein adducts and glutathione turnover can indicate whether quinone traffic is safely resolved. This rescue framing helps explain why similar doses yield divergent outcomes across individuals and disease stages [54].

5.4. Micro-H_2O_2 Relays and Peroxiporins

H_2O_2 is often described as a by-product, yet in many cells it acts as a deliberate second messenger. When silymarin chemistry increases local oxidant tone, very small H_2O_2 pulses can relay information across short distances [55].

These pulses remain useful only if they stay confined, because indiscriminate diffusion would convert signalling into damage. Peroxiporins, aquaporin family channels that facilitate H_2O_2 passage, help define who receives the message, and how quickly it is quenched [56].

At barrier sites, H_2O_2 can also move between neighbouring cells, shaping immune tone and epithelial repair trajectories locally [57].

In epithelial and hepatic contexts, peroxiporin expression and membrane localization can change with inflammation, lipid load, and cytokine exposure. This plasticity may explain why the same silymarin dose yields different redox readouts, and why some tissues show adaptive NRF2 priming while others show stress [58].

The signalling range is set by enzymatic sinks, including peroxiredoxins, catalase, and glutathione peroxidases, which rapidly consume H_2O_2. If these sinks are overwhelmed or inhibited, H_2O_2 expands its radius, oxidises proteins non-specifically, and amplifies mitochondrial and ER stress [59].

Experimental designs should therefore track spatially resolved redox reporters, peroxiporin abundance, and peroxiredoxin oxidation states over time. Pairing these measures with metabolite profiles can indicate whether silymarin creates targeted micro-relays or pushes cells into damaging oxidant spillover [60].

5.5. Hormesis and Safety Windows: When Adaptive Signalling Flips to Injury

Silymarin is best understood through hormesis, where mild electrophile and redox signals provoke protective responses that outlast the initiating chemical pulse. In this regime, NRF2 activation, improved glutathione handling, and restrained inflammation can appear without tissue injury, particularly when baseline oxidative stress is modest [61].

The safety window narrows when exposure becomes continuous, when formulations raise free aglycone, or when deconjugation is amplified in inflamed mucosa and bile niches. Under those conditions, quinone cycling and micro-oxidant relays may exceed enzymatic buffering, producing protein adducts, mitochondrial strain, and stress kinase activation that reinforces inflammation in many tissues that are already metabolically strained [62].

Host factors also shift the tipping point, including steatosis, cholestasis, alcohol use, iron overload, and low antioxidant reserves, which reduce capacity to resolve transient redox perturbations. Drug co-administration can increase risk by changing transporter routing, competing for conjugation, or lowering albumin, increasing unbound exposure even when total concentrations seem unchanged [63].

Operationalizing hormesis means tracking benefit and harm markers, NRF2 targets and autophagy signals alongside aminotransferases, oxidative adducts, and cytokines at matched timepoints. If trajectories show adaptive induction with quick normalisation of redox stress, dosing appears safe, whereas persistent adduct formation or rising injury markers should prompt reduced dosing [64].

5.6. Readouts That Matter: Exposure Biomarkers

Intake-based metrics, such as capsules per day or nominal milligrams, are weak predictors of biology for silymarin. What matters is exposure, which includes which isomers and conjugates appear, their time course, and the unbound fraction that can engage cells [65].

Exposure biomarkers should therefore be prioritised, with targeted quantification of flavonolignan isomers and glucuronide and sulphate panels in plasma, urine, and faeces. Whenever possible, unbound measurements by equilibrium dialysis should accompany totals because binding shifts with inflammation and circulating lipids. Sampling must be time-anchored, with early points that capture absorption, and later points that capture recirculation and microbial processing [66].

Redox readouts should reflect signalling rather than nonspecific oxidative stress, using peroxiredoxin oxidation states, glutathione redox ratios, and induction of NRF2 targets such as NQO1 and HO-1. Coupling these measures to metabolite profiles helps distinguish a brief adaptive pulse from sustained stress [67].

Proteome level endpoints can add specificity, including adductomics, protein carbonyls, and markers of proteostasis such as LC3 turnover and p62 dynamics. When these endpoints are aligned with exposure windows, mechanistic claims become testable and more clinically interpretable [68].

6. Temporal Windows of Activity: Chronopharmacology of Silymarin

Silymarin does not act on a single timeline, because its exposure is shaped by conjugation, transporter routing, and enterohepatic cycling. A chronopharmacology perspective, therefore, helps link what is measured in blood and urine to what cells actually experience in the gut–liver axis. In this framework, the same oral dose can generate distinct signalling opportunities that appear early, recur later, and sometimes persist as low-amplitude tails [69].

The early window, roughly the first two hours after intake, is dominated by circulating conjugates and strong protein binding, yet it can still support localised redox bursts at mucosal and canalicular interfaces. These brief events are well-suited to threshold-based pathway tuning, where NRF2 activation and context-dependent NF-kB modulation are edited rather than fully switched on or off, and this early editing can set the stage for later proteostatic remodelling [70], Figure 8.

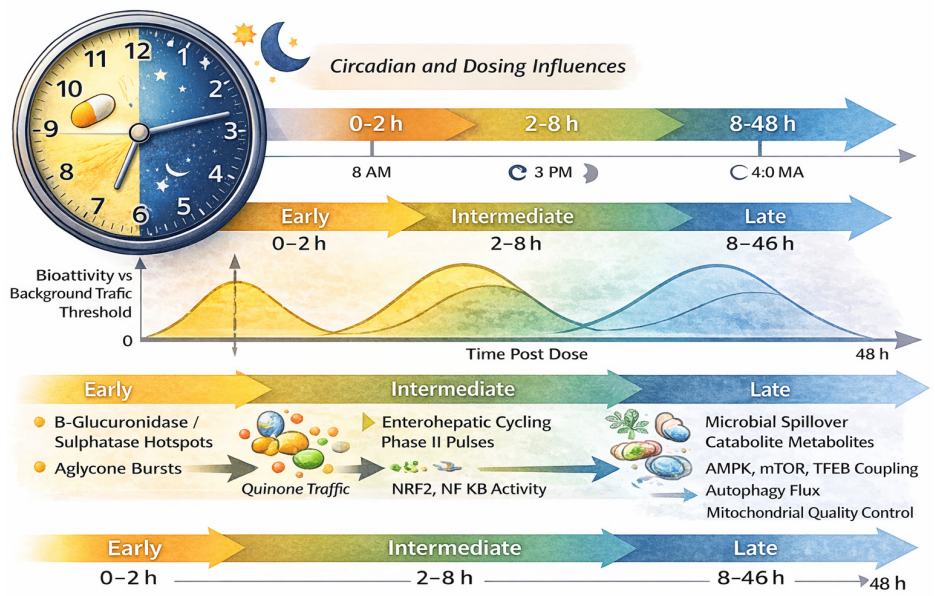

Figure 8. Temporal windows of activity and chronopharmacology of silymarin. Silymarin bioactivity spans early (0 to 2 h), intermediate (2 to 8 h), and late (8 to 48 h) phases that are modulated by the circadian context and meal timing.

6.1. Early Window (0–2 h)

Within the first two hours after dosing, plasma is dominated by glucuronide and sulphate conjugates, usually not aglycones in humans. This early profile reflects rapid enterocyte and hepatocyte conjugation, plus strong protein binding that keeps free parent levels low systemically. Yet tissues facing bile and lumen can experience higher contact, because transporters concentrate conjugates where reactivation can occur locally early [71].

During this window, beta-glucuronidase activity at mucosal interfaces may regenerate small aglycone pulses close to target cells briefly. Because the pulses are spatially confined, they can trigger redox signalling without producing sustained oxidative stress in blood samples alone. A useful framing is threshold editing, where modest electrophile tone nudges sensors, then cellular buffers restore the baseline again quickly afterwards [72].

NRF2 is particularly sensitive, since transient cysteine modifications can release it from repression and upshift antioxidant capacity for several hours. In parallel, NF-κB activity can be tempered, not silenced, supporting inflammation resolution while preserving host defence competence locally. These early changes rarely appear as dramatic endpoint shifts, but they can prime later metabolic and proteostatic responses quite well [73].

For study design, this window rewards dense sampling, including fifteen to sixty minutes, and careful reporting of meal context too. Mechanistic assays should pair metabolite profiling with fast readouts, such as NRF2 target transcripts and peroxiredoxin oxidation states measured concurrently [74].

When interpreted in this temporal frame, conjugate dominant pharmacokinetics becomes an asset, not a limitation, for understanding silymarin in humans [75].

6.2. Intermediate Window (2–8 h)

Between two and eight hours after intake, silymarin exposure often shifts from a single absorption peak to a pattern shaped by redistribution and enterohepatic cycling. Conjugates secreted into bile return to the intestine, where they can persist in mixed micelles and within the mucus layer, creating a sustained reservoir at the epithelial surface. This reservoir matters because it maintains local contact even when plasma levels begin to fall [76].

During the same interval, secondary portal waves can appear when luminal species are reabsorbed and delivered again to the liver. The timing of these waves is influenced by meal-driven bile flow, gastrointestinal motility, and the degree of transporter-mediated recycling. In many designs, these features are missed because sampling schedules assume monotonic decline after the initial peak, and therefore underestimate late signalling opportunity [77].

Biologically, the intermediate window is well suited to repeated low-amplitude pulses, rather than continuous exposure. Local deconjugation can still occur, but the more salient feature may be cumulative editing across multiple small events that gradually bias transcriptional programmes. NRF2-driven capacity, initiated earlier, can stabilise redox tone, while context specific NF-κB modulation may become evident as a dampened inflammatory trajectory rather than an acute suppression [78].

This period is also when formulation differences often emerge clearly, because improved dissolution and micellarization can enlarge the luminal pool and extend the time during which mucosa is exposed. Trials that compare formulations should therefore standardise meals and include sampling at three, five, and eight hours, alongside urine collections. When exposure is tracked with conjugate ratios and unbound estimates, mechanistic readouts become easier to interpret during this intermediate phase [79].

6.3. Late Window (8–48 h)

From eight to forty-eight hours after dosing, the dominant drivers of silymarin related biology are likely indirect and microbiome linked, rather than driven by high systemic peaks. By this stage, host conjugates have been repeatedly delivered to the gut, and microbial enzymes have had time to deconjugate, transform, and catabolize substrates within the lumen and mucus. The resulting chemical space may include regenerated aglycones in small amounts, plus smaller phenolic fragments that can be absorbed and circulate at low concentrations [80].

These late exposures are typically low amplitude, yet they may be biologically meaningful because they coincide with slow processes, such as proteostasis adaptation, mitochondrial quality control, and lipid handling adjustments. Rather than producing dramatic acute changes, late-phase signals may bias set points, lowering basal inflammatory tone, improving antioxidant readiness, or supporting autophagy-related fluxes over days. This fits well with clinical contexts like MASLD, where modest shifts sustained over time can matter more than short-term suppression [81].

Interindividual variability is often largest in this window, because metabotypes differ in beta-glucuronidase activity, in catabolic pathways, and in bile acid ecology. Antibiotic exposure, fibre intake, and baseline inflammation can all reshape these processes, and can alter whether late metabolites appear at all. For that reason, trials that do not capture stool, urine, and at least one late blood draw can miss the main sources of divergence [82].

Experimentally, this window is best studied with repeated measures designs, late sampling at twelve, twenty-four, and forty-eight hours, and paired microbiome and metabolome profiling. When these data are aligned, the late window becomes a tractable component of the mechanism rather than an unexplained tail [83].

6.4. Circadian Dimension

Circadian biology adds an often overlooked layer to silymarin exposure, because the gut–liver axis is not chemically static across the day. Bile secretion follows feeding patterns and endogenous rhythms, and this changes micellar capacity, luminal pH, and the residence time of conjugates at mucosal surfaces. Even when dose is unchanged, the same capsule can meet very different solubilization conditions overall [84].

Hepatic perfusion and metabolic flux also vary with time, which can shift first-pass handling of flavonolignans. Enzymes that drive glucuronidation and sulphation show rhythmic expression and activity in many tissues, and transporter systems that route conjugates toward bile or blood can oscillate as well. These rhythms may move peak times, alter conjugate ratios, and change the balance between portal delivery and canalicular secretion [85].

The microbiome is likewise time-structured, with daily cycles in motility, substrate availability, and microbial gene expression. Beta-glucuronidase activity and bile acid transformations can fluctuate after meals and during fasting intervals, so late window metabolite profiles may differ between morning and evening dosing. This is a practical reason why stool and urine collections should be time-stamped carefully, not treated as generic end-of-day samples [86].

For trial design, circadian considerations argue for consistent dosing time, standardised meals, and reporting of sleep and shift work when relevant. When feasible, crossover studies can test morning versus evening administration while keeping formulation constant. Aligning sampling with expected bile peaks and recirculation waves makes mechanistic readouts more interpretable, and it reduces noise that otherwise masquerades as unexplained individual variability [87].

6.5. Practical Implications: Meal Timing, Dosing Time, Formulation, Co-Interventions

Practical use of silymarin depends on how it is taken, not only on how much is taken. Because dissolution and micellarization are limiting steps, dosing with a meal that stimulates bile flow increases luminal solubilization and mucosal contact. Fat content and meal composition matter, since mixed micelles form differently after foods [88].

Dosing time should be standardised within studies, and considered in routine use, because bile secretion, motility, and hepatic handling fluctuate throughout the day. Morning dosing after breakfast may produce faster early exposure, while evening dosing may prolong luminal residence through slower transit and bile patterns. Consistency is usually far more valuable than chasing an ideal clock time [89].

Formulation is a major determinant, since extracts differ in particle size, excipients, and whether they use phospholipid complexes or other solubilizing strategies. Improved formulations may not simply raise plasma totals, they may reshape routing toward bile and the mucosa, which is the relevant compartment for many proposed mechanisms. Quality control and batch fingerprints should guide product choice in everyday practice [90].

Co-interventions can strengthen or obscure effects, so they should be planned rather than incidental. Dietary patterns that alter bile acids, fibre intake that shifts microbial activity, and drugs that modify transporters or conjugation enzymes can change exposure windows. When trials report these variables, clinical outcomes become more interpretable and reproducible overall [91].

6.6. Time-Resolved Biomarkers and Sampling Strategy

Time-resolved biomarkers are essential for silymarin, because exposure is pulsed and compartmentalised, and single late samples blur absorption, recirculation, and microbial contributions into one uninterpretable average for both clinicians and mechanistic investigators. A practical schedule should anchor early kinetics at 15, 60, and 240 min, capturing peak conjugate formation, initial transporter routing, and the first opportunity for localised reactivation at mucosal surfaces [92].

Sampling should then extend into the intermediate phase, with one point around 6 h and another between 12 and 24 h, to detect secondary portal waves and late signals. These

later draws are where interindividual variability emerges, so they should be paired with standardised meals and clear records of dosing time and sleep timing [93].

Urine provides an integrated view that complements plasma snapshots, because many conjugates and catabolites are cleared renally and accumulate across defined intervals. Collecting urine in timed fractions, such as 0 to 6 h and 6 to 24 h, allows reconstruction of exposure windows without excessive blood draws [94].

Biomarker panels should include isomer-resolved conjugates when feasible, unbound estimates in representative samples, and redox and proteostasis readouts aligned to timing. When transcriptional endpoints are used, they should be sampled close to exposure pulses, so that early NRF2 targets and later autophagy markers are not mistakenly compared [95].

7. Mechanistic Modules: Transcription Factors and Signalling Nodes in Space and Time

Mechanistic interpretation of silymarin improves when signalling nodes are organised by space and timing, rather than by nominal dose alone. Local exposure at mucosal and canalicular interfaces can trigger brief electrophile and redox cues, which are then integrated by transcriptional control circuits.

This section focuses on modules that translate transient chemistry into durable gene programmes, including antioxidant readiness, inflammatory editing, and proteostatic adaptation. We begin with the Keap1 NRF2 ARE axis, because it behaves like a threshold sensor that decodes short pulses, and because it provides measurable targets that map to human-relevant exposure, Figure 9.

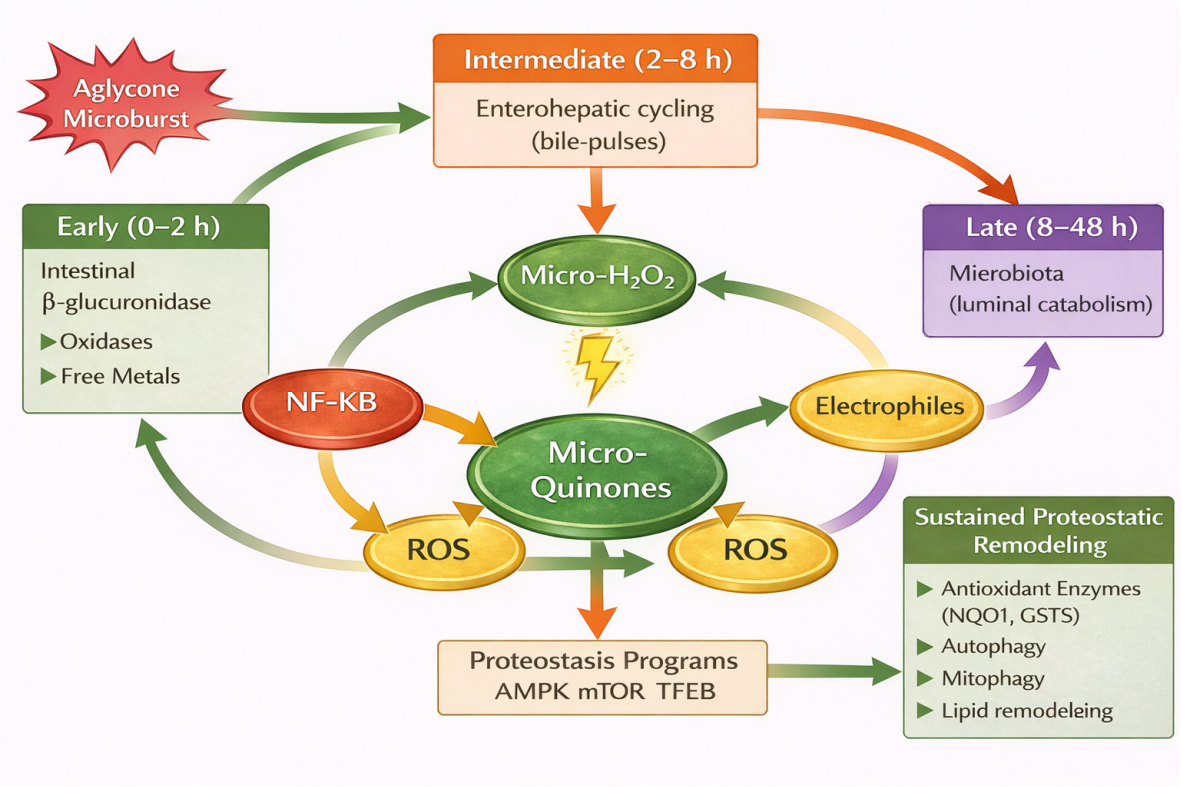

Figure 9. Mechanistic modules of silymarin signalling in space and time. Spatially gated exposure along the gut–liver axis generates transient redox and electrophile cues that are decoded by interconnected signalling nodes across early, intermediate, and late temporal windows.

7.1. Keap1-NRF2-ARE Thresholds and Electrophile Decoding

The Keap1, NRF2, and ARE pathway offers a practical lens for silymarin biology, often when plasma aglycones seem negligible. Keap1 functions as an electrophile-sensitive repressor, using reactive cysteines to control NRF2 degradation under basal conditions. When those cysteines are modified, NRF2 stabilises, enters the nucleus, and activates ARE-driven transcriptional programmes [96].

This system behaves like a threshold device, because many redox events are buffered and then erased without durable consequences. Short electrophile pulses can be reversed quickly, whereas slightly larger pulses cross kinetic limits and prolong NRF2 activity for hours. Silymarin fits this logic because conjugates dominate circulation, yet local deconjugation can generate brief aglycone and quinone exposure near mucosa or canalicular membranes [97].

Electrophile identity matters, because Keap1 does not respond equally to every modifier, and different cysteines encode different outcomes. Quinone prone intermediates often yield reversible adducts that support adaptation, particularly when NQO1 and glutathione systems remain sufficient. Cell type and disease state shift the apparent threshold, since steatotic liver and inflamed mucosa begin with altered thiol tone and basal NRF2 activity [98].

Repeated microbursts may sum through transcriptional memory, because NRF2 target proteins persist beyond the initiating chemistry. This persistence helps align early redox edits with later proteostasis remodelling, including turnover of damaged proteins. It also explains why single endpoint assays can miss benefits when sampling occurs after recovery [99].

For rigorous interpretation, studies should quantify NRF2 target induction alongside exposure markers, rather than inferring activation from downstream outcomes alone. Useful endpoints include NQO1, HO-1, GCLC, and timed measures of peroxiredoxin oxidation that report both signal amplitude and recovery. When collected across early and intermediate windows, these readouts test whether spatially gated electrophile pulses truly reprogram antioxidant capacity in humans [100].

7.2. NF-κB/IKK Modulation as Context-Dependent Inflammatory "Editing"

NF-kB signalling is often described as a master inflammatory switch, yet in vivo it behaves as a context editor. The IKK complex integrates cytokines, microbial products, oxidative tone, and metabolic stress, then shapes transcriptional outputs over time. Because silymarin exposure is spatially gated, its impact on this pathway is expected to be conditional and compartment-specific [101].

During the early post-intake window, plasma is dominated by conjugates, while mucosal surfaces may experience brief aglycone regeneration. In that setting, silymarin may reduce the amplitude or duration of NF-kB pulses, without erasing host defence responses. This pattern fits an editing model, where resolution programmes proceed more easily, and collateral tissue damage is limited [102].

In the intermediate window, enterohepatic recirculation can re-deliver conjugates to the gut–liver axis, sustaining low level inflammatory editing again locally. This is most relevant in MASLD and alcohol-related injury, where baseline NF-kB tone is elevated yet plastic still measurable [103].

Several mechanisms can converge on IKK and its upstream regulators in a redox sensitive manner. Transient electrophile events can alter thiol-based control nodes, kinase and phosphatase balance can shift, and membrane signalling platforms can reorganise as lipid handling improves. Importantly, these effects can diverge by cell type, since

epithelial repair may benefit from restrained signalling while immune cells must retain rapid responsiveness [104].

For clinical translation, studies should not look only for blanket cytokine suppression after supplementation. Instead, trials should verify exposure and sample kinetics, measuring NF-kB target transcripts, cytokine trajectories, and resolution markers across early and intermediate timepoints [105].

7.3. AMPK-mTOR-TFEB Axis: Metabolic-Proteostatic Coupling

The AMPK-mTOR-TFEB axis links nutrient sensing to proteostasis, and it offers a coherent framework for interpreting silymarin effects beyond simple antioxidant claims in humans. AMPK is activated when cellular energy balance tilts, and modest redox signals can reinforce this shift by tuning upstream kinases and phosphatases locally as well. In hepatocytes and mucosal immune cells, AMPK activation can suppress mTORC1 activity, easing the brake on lysosomal programmes controlled by TFEB over time thereafter [106].

This matters for silymarin because early conjugate-dominated exposure may prime stress signalling, while later recirculation supports sustained metabolic recalibration during daily feeding cycles. TFEB activation promotes autophagy, lysosomal biogenesis, and lipid droplet clearance, outcomes that align with liver phenotypes targeted in MASLD studies in clinical practice now. However, TFEB responses are sensitive to compartment, since intestinal epithelium may prioritise barrier repair, whereas macrophages may shift cytokine outputs with distinct kinetic profiles [107].

To avoid overinterpretation, experiments should match concentrations to unbound human exposure, and they should include transporter competent models that preserve routing across cell layers. Time-resolved sampling is equally important, because AMPK activation can occur within hours, while TFEB-driven remodelling often requires repeated exposures and recovery periods [108].

Useful biomarkers include phosphorylation of AMPK targets, mTORC1 readouts such as pS6, and transcription of lysosomal genes under TFEB control in paired timepoints too. When these measurements are integrated with metabolite profiles and meal timing, the AMPK-mTOR-TFEB axis becomes a testable mechanism rather than a metaphor for silymarin biology [109].

7.4. PPAR Modules

PPAR signalling provides a useful bridge between lipid handling and inflammation, which is exactly where silymarin is most often applied clinically. PPAR alpha supports hepatic fatty acid oxidation and ketogenesis, and PPAR gamma shapes insulin sensitivity and macrophage phenotype, while PPAR delta integrates energy use across tissues. These nuclear receptors respond to lipid-derived ligands and cofactor states, so they are sensitive to the metabolic context of the gut–liver axis [110].

Silymarin exposure may influence PPAR modules indirectly by reducing inflammatory tone and oxidative stress that otherwise suppresses oxidative metabolism. In a bile-first framework, repeated delivery of conjugates and locally regenerated aglycones could bias hepatocyte and macrophage programmes toward improved lipid flux, without requiring high systemic aglycone levels. This fits a model where modest signalling edits accumulate across meals and recirculation cycles, rather than acting as acute pharmacological switches [111].

PPAR activity also interacts with bile acid signalling and microbiome-derived metabolites, creating a web of feedback loops. When bile composition changes and mucosal inflammation resolves, microbial ecology can shift, altering short-chain fatty acids and secondary bile acids that themselves modulate metabolic and inflammatory pathways. This

complexity helps explain heterogeneity in MASLD outcomes, where diet and microbiome differences can dominate variance [112].

To make PPAR claims credible, studies should measure pathway outputs, not only receptor expression. Useful readouts include fatty acid oxidation genes, lipid droplet handling markers, and macrophage polarisation signatures in time-resolved designs. Pairing these endpoints with metabolite profiling and careful reporting of diet and formulation strengthens the mechanistic chain from silymarin exposure to lipid inflammation integration [113].

7.5. SIRT1/FOXO/PGC-1α: Mitochondrial and Stress-Response Remodelling

SIRT1, FOXO factors, and PGC-1α form a tightly connected network that links nutrient state, redox tone, and mitochondrial quality. In hepatocytes and immune cells, this axis helps decide whether stress signals become damaging or are translated into durable adaptive remodelling that improves resilience over time in vivo [114].

SIRT1 activity depends on NAD+ availability, which is shaped by energy flux, inflammation, and circadian timing. When silymarin exposure nudges cellular redox balance and lowers inflammatory pressure, NAD+ pools may be preserved, supporting deacetylation of FOXO proteins and PGC-1α. This can promote antioxidant enzymes, mitochondrial biogenesis programmes, and improved fatty acid handling, especially in metabolically strained liver tissue [115].

FOXO transcription factors also often integrate transient oxidant pulses, converting short-lived signals into gene expression patterns that enhance repair, autophagy support, and glucose homeostasis. PGC-1α amplifies these effects by coordinating nuclear and mitochondrial gene expression, and by improving respiratory capacity. These changes are unlikely to follow a single-peak concentration, and they fit better with repeated low-amplitude exposure across enterohepatic cycles [116].

Mechanistically credible studies should measure NAD+-related markers, SIRT1-dependent acetylation states, and PGC-1α target transcripts alongside time-resolved metabolite profiles. Readouts should be collected at early and late windows, because transcriptional priming can precede mitochondrial remodelling by many hours. When this timing is respected, the SIRT1, FOXO, PGC-1α axis becomes a plausible bridge between spatial exposure and clinically relevant metabolic improvement [117].

8. Proteostasis Outcomes: From Transient Chemistry to Durable Remodelling

Proteostasis outcomes are where transient redox chemistry becomes biologically durable, especially in metabolically stressed liver and barrier tissues. Short signalling pulses can reshape clearance capacity, not by forcing constant autophagy, but by gradually improving lysosomal throughput and organelle quality control. This matters for clinical translation, because steatosis, inflammation, and toxin exposure all create protein and lipid burdens that accumulate over weeks and months [118]. In this section, we therefore shift from pathway triggers to functional consequences, asking whether silymarin exposure can plausibly enhance degradation, recycling, and repair in the compartments it most consistently reaches. We begin with TFEB because it coordinates lysosomal biogenesis and sets the ceiling for downstream autophagy and mitophagy efficiency, Figure 10.

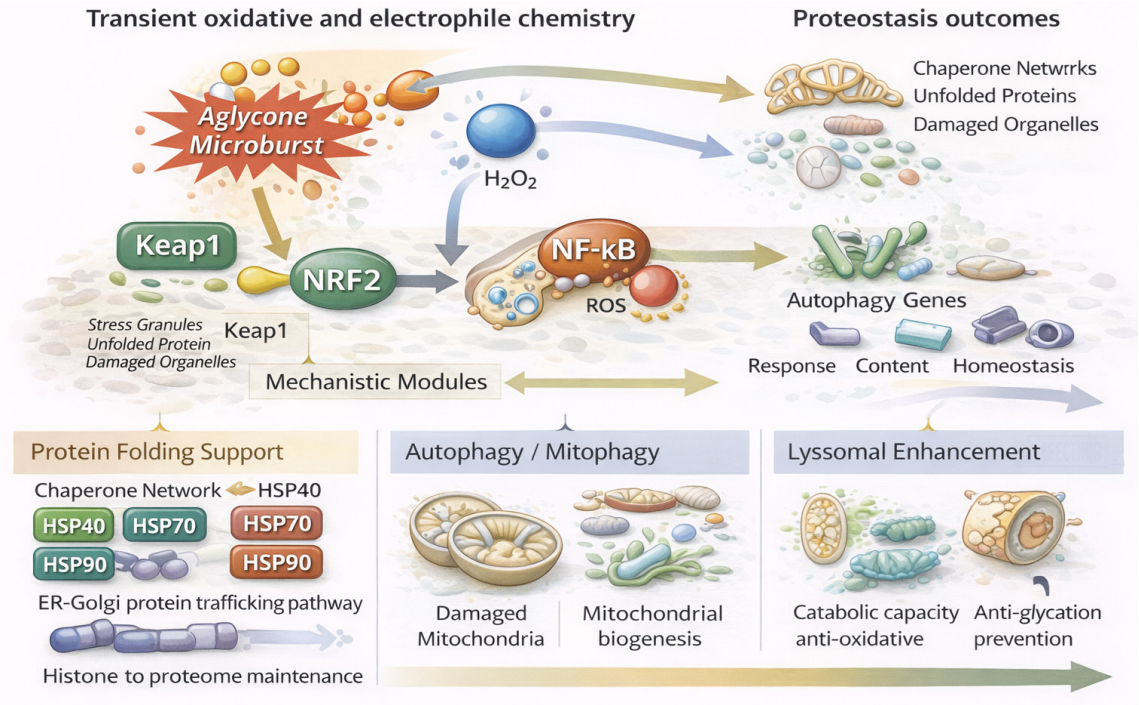

Figure 10. Proteostasis outcomes from transient chemistry to durable remodelling. Brief, spatially confined aglycone microbursts and micro-H_2O_2 relays trigger reversible activation of Keap1-NRF2 and context-dependent NF-κB signalling, converting short-lived redox events into sustained proteostatic responses. Downstream engagement of chaperone systems, autophagy and mitophagy pathways, and TFEB-driven lysosomal enhancement supports clearance of damaged proteins and organelles, promoting long-term cellular homeostasis and stress resilience.

8.1. TFEB Programmes and Lysosomal Biogenesis

TFEB is a central transcriptional regulator of lysosomal biogenesis, coordinating the CLEAR gene network that expands lysosome number, acidity, and degradative capacity. In hepatocytes and intestinal barrier cells, these programmes are crucial because progressive lipid overload and chronic inflammation frequently converge on defective cargo clearance [119].

Under nutrient-rich conditions, mTORC1 phosphorylates TFEB and retains it in the cytosol, preventing lysosomal gene induction. When energy stress activates AMPK, and when upstream signals reduce mTORC1 activity, TFEB dephosphorylation promotes nuclear translocation and sustained transcriptional output. This coupling provides a realistic route by which early silymarin signalling pulses can translate into later proteostatic remodelling, without requiring continuous systemic aglycone exposure [120].

A bile-first routing model further supports this timing, since conjugates are enriched in bile and repeatedly delivered to mucosa through enterohepatic cycling. Within mucus and inflamed niches, intermittent deconjugation can regenerate small aglycone pulses that act as brief electrophile and redox triggers near target cells. Repetition of these confined events can bias TFEB-dependent transcription over days, increasing lysosomal competence for lipid droplet turnover, damaged protein disposal, and mitochondrial renewal processes that depend on lysosomal throughput [121].

Mechanistic work should measure TFEB localization dynamics, alongside lysosomal gene signatures, and quantify how long transcriptional changes persist after exposure has fallen. Functional endpoints can include cathepsin activity, lysosomal acidity proxies, substrate degradation assays, and autophagy flux readouts collected at matched timepoints. In clinical cohorts, extracellular vesicle markers may offer a feasible window into lysosomal status [122].

Experimental systems should preserve transporter directionality, physiological protein binding, and time-resolved sampling across early and late windows, so TFEB responses are not mistaken for artefacts of chronic supraphysiological dosing [123].

8.2. Autophagy Flux and Cargo Selection

Autophagy is frequently reported as a marker, yet its meaning depends on flux, which is the rate of cargo delivery to lysosomes and degradation. For silymarin, flux matters because transient signalling pulses are more likely to retune turnover capacity than to force a permanent on state of autophagosome formation in every cell [124].

LC3 processing and p62 dynamics remain the most accessible readouts, although they are easy to misinterpret without inhibitors and time matching. LC3-II accumulation can indicate increased autophagosome biogenesis, or impaired clearance, and p62 can fall with enhanced flux, or rise when oxidative stress increases its expression. Rigorous assays therefore pair LC3 and p62 with lysosomal blockade, for example bafilomycin or chloroquine, and quantify the delta across defined exposure windows [125].

Cargo selection is equally important, because clinical relevance often comes from selective autophagy, not bulk recycling. Lipophagy can influence hepatic lipid droplet burden, mitophagy supports mitochondrial quality control, and xenophagy shapes mucosal inflammation through pathogen handling. These routes rely on adaptor proteins, ubiquitin tagging, and membrane lipids that are sensitive to redox tone and to AMPK-mTOR-TFEB signalling [126].

Experimental systems should preserve transporter routing and physiological protein binding, then sample at early and late points to capture both priming and remodelling. Endpoints can include tandem fluorescent LC3 reporters, degradation of long-lived proteins, and proteomic signatures of selective cargo, interpreted alongside metabolite profiles and bile-linked timing markers. In human studies, paired urine metabolites can anchor exposure to flux readouts [127].

8.3. Mitophagy and Mitochondrial Quality Control

Mitophagy is the selective autophagic removal of damaged mitochondria, and it safeguards energy metabolism in hepatocytes, enterocytes, and tissue macrophages. In MASLD and chronic mucosal inflammation, lipid overload and persistent oxidant tone increase mitochondrial injury, while turnover pathways can become sluggish. When dysfunctional organelles accumulate, cells drift toward inflammatory signalling, impaired beta oxidation, and reduced stress tolerance [128].

Silymarin is unlikely to stimulate mitophagy by acting as a strong mitochondrial toxin, instead it may tune the decision points that favour repair or disposal. Spatially confined electrophile and redox pulses, generated near bile-facing and mucosal interfaces, can activate AMPK signalling and lower mTORC1 activity, which supports TFEB-linked lysosomal competence over time. Improved lysosomal capacity matters, because labelled mitochondria must be delivered and degraded efficiently, not merely recognised [129].

At the organelle level, pathways involving PINK1 and Parkin, BNIP3 and NIX, and FUNDC1 translate membrane potential loss, oxygen tension cues, and kinase balance into ubiquitin tags and receptor signals. Credible assays should use mitophagy reporters such as mito-Keima, or tandem fluorescent mitochondrial constructs, and interpret them alongside LC3 recruitment, p62 turnover, and flux controls with lysosomal inhibitors. Without flux validation, apparent increases in mitophagy markers can reflect blocked clearance rather than accelerated organelle disposal [130].

In humans, exposure-verified metabolite profiling should be paired with indicators of mitochondrial stress, including acylcarnitines, FGF21, and cell-free mitochondrial DNA,

collected at matched early and late timepoints. Consistent meal timing and activity logs reduce noise because mitochondrial turnover follows daily rhythms. These linked measurements can separate signalling priming from genuine organelle renewal across diverse clinical settings [131].

8.4. ER Stress/UPR as a Proteostasis Interface

The endoplasmic reticulum is a central proteostasis hub, and ER stress is a common endpoint in fatty liver disease, alcohol-related injury, and inflammatory barrier dysfunction. When lipid load, calcium imbalance, or oxidant tone disrupt folding, the unfolded protein response coordinates adaptation through three major arms, PERK, IRE1, and ATF6. These pathways recalibrate translation, chaperone capacity, and degradation routes, and they also intersect directly with inflammatory signalling [132].

Silymarin-related effects on the UPR are likely to be indirect and context dependent, because the dominant systemic species are conjugates, and exposure is spatially gated. In a bile-first model, localised redox microbursts at mucosal or canalicular interfaces could reduce inflammatory pressure and improve redox buffering, which would lower the burden of misfolded proteins entering the ER. Over time, improved mitochondrial function and lipid handling may also reduce lipotoxic stress that drives ER dysfunction [133].

However, ER stress can also be amplified if electrophile tone is excessive or if detoxification capacity is limited. Quinone chemistry and glutathione depletion can perturb ER redox balance, disrupt disulphide formation, and intensify PERK signalling. This is why a hormetic framing is essential, and why safety windows should be assessed with proteostasis endpoints rather than relying only on liver enzymes [134].

Experimental work should track UPR-branch-specific markers, such as XBP1 splicing, ATF4 and CHOP induction, and ATF6 targets, alongside autophagy flux and metabolite profiles in time-resolved designs. In clinical studies, pairing these markers with exposure-verified conjugate panels can clarify whether silymarin supports adaptive resolution or simply coincides with the variable disease course [135].

8.5. Proteasome/Chaperones and Handling of Oxidised/Adducted Proteins

Proteasomes and molecular chaperones provide an energy-dependent defence against damaged proteins, often before autophagy becomes dominant. In liver and intestinal cells, oxidative pressure and lipid stress increase misfolding, carbonylation, and covalent adduct formation, which must be triaged quickly to preserve signalling specificity and membrane function [136].

Silymarin related redox signalling can influence this triage in two opposite directions, depending on dose and context. Mild electrophile pulses may raise NRF2 activity and increase expression of proteasome subunits and heat shock proteins, improving clearance capacity while reducing inflammatory amplification. When buffering is limited, repeated quinone traffic can overload ubiquitination and stall the proteasome, leaving adducted proteins to aggregate [137].

Methodologically, studies should measure proteasome function, not only transcript levels, because activity can fall despite apparent induction. Assays of 20S and 26S catalytic rates, ubiquitin conjugate burden, and chaperone induction, such as HSP70 and HSP90, should be aligned with time-stamped metabolite profiles. Parallel quantification of protein carbonyls and targeted adductomics can clarify whether signalling remains reversible [138].

Clinically, these endpoints may explain why some trials show improved liver enzymes without durable metabolic change. If proteasome and chaperone capacity rises early, later remodelling becomes feasible, whereas persistent adduct accumulation should be interpreted as a warning sign that the hormetic window has been exceeded. In practice,

peripheral blood mononuclear cells can provide accessible proteostasis readouts longitudinally [139].

8.6. Experimental Toolbox

The mechanistic claims developed throughout this section carry an implicit experimental demand: the models used to test them must reproduce the very features that make silymarin biology distinctive. Conventional monolayer cultures cannot fulfil this requirement because they lack the directional transport architecture that governs conjugate secretion and compartment-specific enrichment. Polarised intestinal epithelia, hepatocyte sandwich cultures, and gut-liver microphysiological chips are not simply more elaborate alternatives; they are the minimum platform capable of generating apical and basolateral gradients that reflect the bile-facing and lumen-facing exposures observed in vivo [140].

The temporal structure of silymarin signalling imposes an equally specific demand on omics study design. Because the compound delivers pulsed rather than sustained signals, a single endpoint measurement conflates pharmacologically distinct phases. Sampling at 15, 60, and 240 min captures the priming window when rapid conjugate waves reach their targets, whereas the 6 to 24 h interval reflects slower transcriptional and proteostatic remodelling. Separating these phases is essential for interpreting whether autophagy flux and lysosomal gene induction represent an adaptive response to early exposure or a downstream consequence of more sustained redox editing [141].

Establishing which proteins are genuinely engaged during physiologically relevant exposure requires tools that go beyond expression profiling. Cellular thermal shift assays and thermal proteome profiling report stability changes across the proteome without requiring prior target nomination, which is particularly valuable for silymarin given the ongoing debate about its primary molecular interactors. Integrating stability data with measurements of unbound conjugate concentrations and conjugate speciation panels allows the analyst to distinguish targets that are physically accessible under realistic exposure conditions from those that appear relevant only at supraphysiological concentrations [142].

Because the most consequential signalling events described in this section are spatially confined to specific cell neighbourhoods, verification cannot rely on bulk tissue measurements alone. Redox reporter imaging, spatial transcriptomics, and MALDI imaging mass spectrometry each offer the spatial resolution needed to confirm that metabolite accumulation and transcriptional responses are co-localised rather than coincidental. Combining these spatial readouts with rigorous exposure verification, standardised meal protocols, and predefined sampling windows is what ultimately converts mechanistic proposals into reproducible and clinically interpretable evidence [143].

9. Human Evidence and Clinical Translation: What Works, for Whom, and Why

Clinical translation is where mechanistic elegance meets the messy reality of diet, adherence, and comorbidity. Silymarin trials span diverse liver conditions, yet results are hard to compare because products, doses, and endpoints differ widely across studies [144].

A spatial pharmacology perspective predicts that benefits will track exposure-verified metabolites and time windows, not capsule counts alone. This section, therefore, synthesises human evidence with attention to formulation, dosing context, baseline phenotype, and whether outcomes align with plausible redox and proteostasis mechanisms, Figure 11.

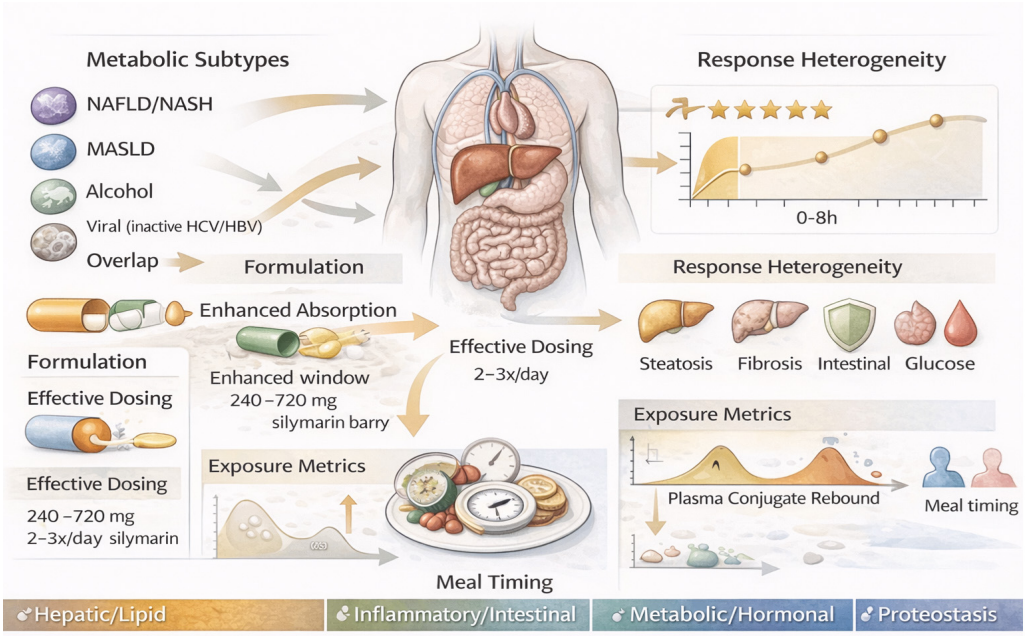

Figure 11. Human evidence and clinical translation: what works, for whom, and why. Clinical outcomes with silymarin vary across MASLD or NAFLD and alcohol-associated liver disease cohorts because effective exposure is shaped by formulation, dosing frequency, and meal or circadian timing, rather than by nominal milligram dose alone.

9.1. MASLD and NAFLD

Clinical interest in silymarin for MASLD and NAFLD reflects a plausible mechanistic fit, yet trial results remain uneven because endpoints, duration, and exposure verification vary widely. These conditions evolve slowly, and brief interventions can miss meaningful change even when molecular pathways shift. A spatial pharmacology view also predicts that benefits may concentrate in the gut–liver axis, rather than appearing as systemic signals [145].

Endpoint choice is a major source of heterogeneity across studies in practice. Many trials rely on aminotransferases, which fluctuate with weight change, alcohol intake, and intercurrent illness, and they do not reliably track steatosis or fibrosis. More informative outcomes include MRI of liver fat, elastography, and composite scores that reflect inflammation and fibrotic risk. When biopsies are available, activity scores and ballooning link more directly to cellular stress programmes [146].

Duration and dosing strategy matter because the most plausible mechanisms involve gradual remodelling. Improved lipid handling, dampened inflammatory tone, and better proteostasis through AMPK, TFEB, and autophagy-related programmes usually require repeated exposures over weeks. Trials shorter than twelve weeks may capture early signalling edits, while failing to move imaging endpoints. Consistent dosing time, standardised meals, and metabolite profiling help separate pharmacology from lifestyle noise [147].

Finally, MASLD populations are intrinsically diverse, and that diversity can dominate treatment effects. Baseline insulin resistance, bile acid patterns, microbiome metabotypes, and comedications can redirect conjugate routing and local reactivation, producing responders and non-responders within one cohort. Stratifying by metabolic phenotype and microbiome features, combined with time-resolved metabolite panels, can align clinical outcomes with the exposure windows that silymarin is most likely to influence [148].

Three clinically relevant subgroups deserve specific attention. First, patients with type 2 diabetes and insulin resistance exhibit altered bile acid profiles, reduced hepatic OATP transporter expression, and higher baseline oxidative tone, all of which can shift silymarin

routing away from bile and toward portal blood, potentially changing the time windows and tissue compartments where signalling occurs. In this subgroup, AMPK-mTOR-TFEB coupling is also tonically suppressed by chronic hyperinsulinaemia, which may blunt the proteostatic remodelling that silymarin is proposed to facilitate. Trials in diabetic MASLD should therefore report fasting insulin, HOMA-IR, and bile acid profiles alongside metabolite-resolved exposure. Second, patients with obesity have expanded adipose tissue that can sequester lipophilic species and alter apparent distribution volumes. Higher body mass also correlates with greater gut permeability and higher baseline β-glucuronidase activity, suggesting that microburst amplitude may be elevated in this subgroup; this could amplify both benefit and hormetic risk. Dose-finding studies that include unbound exposure measurements stratified by BMI are needed. Third, elderly patients present a distinct pharmacological landscape: reduced Phase II enzyme activity lowers conjugation rates, altered microbiome composition (reduced diversity, higher opportunistic GUS activity) changes late-window catabolite profiles, polypharmacy increases transporter competition, and lower albumin raises free fractions. Available evidence from mixed-age cohorts does not allow conclusions specific to older adults, and dedicated pharmacokinetic studies in patients over 65 years are an important unmet need.

9.2. Alcohol-Associated Liver Disease and Inflammatory Liver Conditions

Alcohol-associated liver disease presents a different exposure and stress landscape than MASLD, and this may change how silymarin performs clinically. Ethanol metabolism increases acetaldehyde burden, disrupts mitochondrial function, and elevates oxidative and inflammatory tone, often alongside altered bile flow and gut permeability. These features concentrate injury at the gut–liver interface, where a bile-first botanical mixture could plausibly exert local effects [149].

In this context, the most defensible expectation is not a dramatic reversal of established fibrosis, but a moderation of inflammatory trajectories and a support of stress handling systems. NRF2-related induction can improve glutathione economy and detoxification readiness, while context-specific editing of NF-kB activity may reduce collateral cytokine amplification without suppressing immune defence. Proteostasis pathways, including autophagy and lysosomal capacity, are also relevant because alcohol impairs organelle turnover and increases damaged protein accumulation [150].

Clinical studies in alcohol-related disease are often confounded by abstinence patterns, nutritional status, and concurrent medications, all of which can dwarf supplement effects. Endpoints such as aminotransferases can improve rapidly with reduced drinking, so interpretation requires careful tracking of intake, and ideally objective biomarkers of alcohol exposure. Longer follow up that includes imaging measures, inflammatory markers, and metabolite verified adherence is more informative [151].

Inflammatory liver conditions beyond alcohol, including cholestatic states and drug related injury, raise additional safety considerations. Transporter inhibition, impaired conjugation, and hypoalbuminemia can raise unbound exposure unexpectedly. Trials should therefore include exposure profiling and safety monitoring that captures redox stress and proteostasis strain, not only routine liver chemistry panels [152].

9.3. Other Indications + Safety: Why Designs Read "Null"

Silymarin has been studied beyond fatty liver disease, including drug-induced liver injury, cholestatic disorders, and metabolic risk states. Results appear mixed, not because the biology is absent, but because designs blur exposure, context, and outcomes. This is particularly relevant when participants use diverse botanicals, alcohol, and analgesics [153].

Many null trials simply reflect formulation limits and dosing choices that vary across studies. Poorly solubilized products can deliver inconsistent luminal availability, while improved complexes may shift routing toward bile and mucosa without raising plasma totals. If dosing time and meals are uncontrolled, enterohepatic pulses vary across participants and mechanistic readouts are sampled at the wrong moment [154].

Endpoints are often mismatched to plausible mechanisms and to realistic intervention lengths. Short interventions that measure only aminotransferases, or broad symptom scores, may miss changes in inflammatory tone, redox buffering, and proteostasis programmes that evolve gradually. Conversely, expecting rapid fibrosis reversal sets an unrealistic bar, especially when background lifestyle changes dominate variance [155].

Three illustrative cases clarify how these design problems translate into specific null findings, and how they could be corrected. (i) Saller et al., 2008, reviewed RCTs in drug-induced liver injury and found no consistent benefit from silymarin across studies using 280–800 mg/day [156]. However, none of the included trials reported conjugate profiles or sampling beyond 4 h post-dose, meaning that enterohepatic recirculation waves (expected at 4–8 h) were systematically missed, and formulation differences were not controlled. A redesigned trial would standardise the product (batch fingerprint, silybin A/B ratio reported), include 0, 1, 2, 4, 8, and 24 h sampling, and use faecal calprotectin to stratify participants by mucosal inflammation status before randomisation. (ii) In a Cochrane review of silymarin in alcoholic liver disease Rambaldi et al., 2005 concluded that evidence was insufficient, partly because trials differed in abstinence verification [157]. Confounding by ongoing alcohol use inflates aminotransferase variance, obscuring any supplement effect. Improvement requires mandatory objective alcohol biomarkers (urinary EtG-glucuronide, serum phosphatidylethanol) collected at each visit, with analysis pre-specified as intention-to-treat in completers who confirm abstinence. (iii) A pilot study in cholestatic patients, it was found no reduction in serum bile acids after eight weeks. Cholestasis impairs the biliary secretion of conjugates, thereby disrupting the bile-first routing that underpins silymarin's proposed mechanism; expecting the same effect as in MASLD ignores this fundamental pharmacokinetic difference. Future trials in cholestatic disease should shift endpoints to mucosal inflammation markers, intestinal barrier integrity (zonulin, faecal calprotectin), and redox biomarkers rather than serum bile acids, and should include transporter genotyping (ABCB11, ABCC2) as a stratification variable.

10. Determinants of Variability and Precision Use

Determinants of variability are central to silymarin research, because the same nominal dose can yield different exposure routes, time windows, and signalling consequences across individuals. These differences often arise from factors that are rarely standardised, including product composition, solubilization, meal context, microbiome activity, and comedication. A precision use perspective, therefore, treats heterogeneity as informative, not as nuisance, and it asks which variables predict responders and which variables predict null outcomes [158].

We begin with formulation and product quality, because this is the first point where variability enters, and because it shapes dissolution, micellarization, and transporter-mediated routing before any biological pathway is engaged. Clear reporting of product identity and verified chemical profiles is therefore a prerequisite for meaningful comparisons across trials and laboratories, Figure 12.

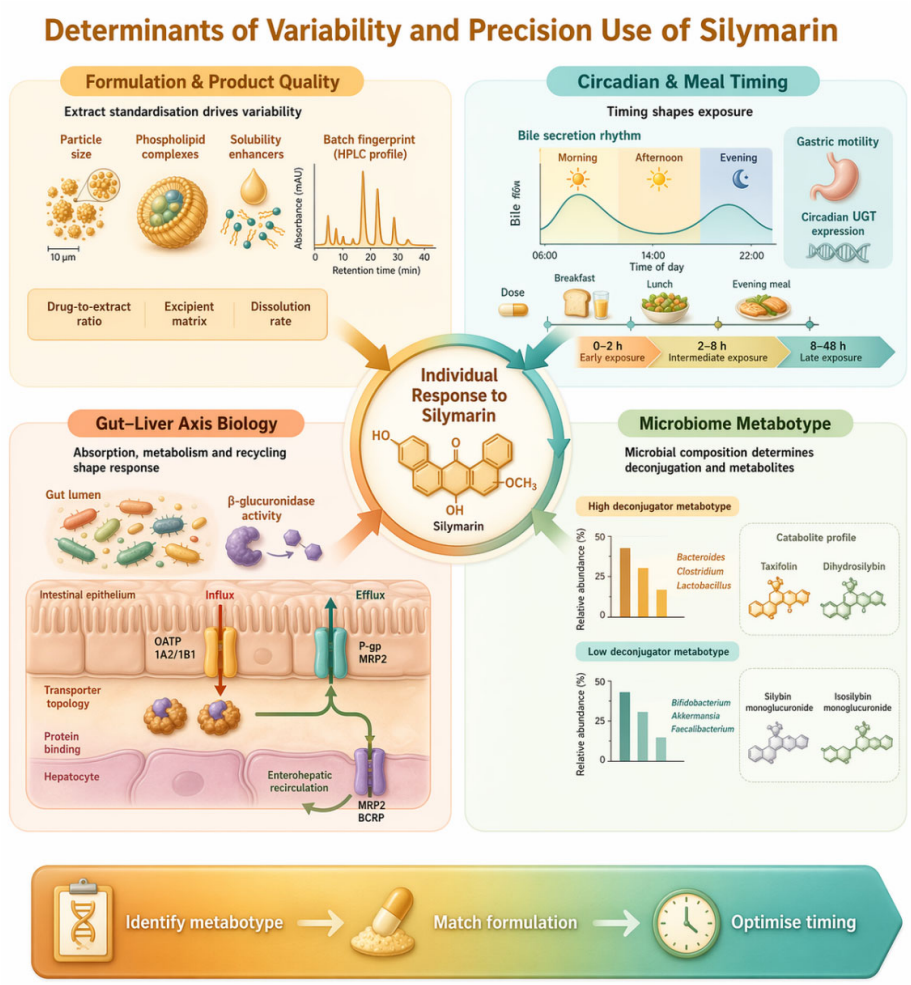

Figure 12. Determinants of variability and precision use of silymarin. Interindividual response to silymarin arises from the interaction of formulation-dependent exposure, circadian timing, and gut–liver axis biology, including bile ecology, transporter topology, protein binding, and microbiome driven deconjugation.

10.1. Formulation and Product Quality: Why "Silymarin" Products Differ

Silymarin products differ because the label term refers to a botanical extract class, not a single molecule, and regulators often allow broad compositional ranges that remain clinically meaningful. Variability begins with plant genetics, cultivation conditions, harvest maturity, drying, and storage, which can shift oxidation state, moisture, and the distribution of flavonolignan isomers before extraction begins [159].

Extraction and purification decisions then amplify divergence, since solvent polarity, temperature, and adsorption steps change the relative abundance of silybin A and B, isosilybins, silychristin, silydianin, and taxifolin. Some manufacturers enrich a narrow fraction to raise the reported percent total silymarin, while others retain broader phenolic companions that may influence micellar behaviour, conjugation patterns, and downstream transporter handling [160].

Formulation technology introduces another layer, because poor aqueous solubility limits its dissolution and mixed micelle incorporation in the intestine, especially under variable bile flow and meal composition. Phospholipid complexes, micronization, solid dispersions, and bile-compatible excipients can increase luminal availability, yet they can also reshape routing, changing the balance between mucosal reservoirs, bile secretion, and portal delivery. These differences are exaggerated by bioavailability enhancers, and claims

of superiority should be supported by metabolite-resolved pharmacokinetics, including unbound estimates, rather than by peak plasma values alone [90].

Quality control depends on analytical rigour and transparency, and many clinical reports still provide only a single percentage value, without batch fingerprints, isomer resolution, or validated calibration against authentic standards. Independent testing occasionally finds label mismatches, oxidation products, or unwanted residues, so trials should document manufacturer, lot number, stability, and contaminant screening, and should interpret outcomes in light of verified chemical exposure [4].

10.2. Interindividual Factors

Interindividual variability is a central feature of silymarin translation, because the same oral dose can produce different exposure routes and signalling windows across people. A major source is the microbiome, which determines metabotypes through differences in beta-glucuronidase and sulphatase activity, bile acid transformations, and luminal redox ecology. These differences influence whether conjugates remain inert passengers or become substrates for local reactivation and downstream catabolite generation [161].

Host metabolism and transport add another layer, since UGT and SULT activity shapes the conjugate panel that circulates and enters bile. Genetic variants, liver function, and concomitant medications can shift these pathways, altering how quickly aglycones disappear and which conjugates dominate. Transporter expression and function, both in enterocytes and hepatocytes, determine whether metabolites are recycled apically, exported to portal blood, or secreted into bile, and these traits vary with genotype, diet, and disease state [162].

Inflammation often amplifies variability by changing plasma protein binding, transporter regulation, and mucosal permeability. Hypoalbuminemia and elevated free fatty acids can raise unbound fractions, increasing cellular access even when total concentrations look unchanged. In inflamed niches, redox buffering is reduced, so the same local microburst can have stronger signalling consequences, or cross into injury if capacity is limited [163].

Age and sex influence bile flow, enzyme expression, body composition, and hormonal regulation, and they are plausible modifiers of time windows. Older adults often have altered microbiome composition and comorbidity medication burden, while sex-related differences in conjugation and transporter regulation can affect exposure patterns. Trials should therefore collect these covariates systematically, and whenever possible stratify or adjust analyses, rather than treating variability as unavoidable noise [164].

10.3. Herb–Drug Interaction Logic

A useful way to think about herb–drug interactions with silymarin is to start from routing and metabolism, rather than from long lists of speculative pairings. In most people, silymarin exposure is dominated by conjugated metabolites that are actively transported, and this makes transporter systems and conjugating enzymes the most plausible interaction points [165].

The first question is whether a co-administered drug shares uptake or efflux pathways in the gut or liver. If both substrates compete for the same apical efflux transporters in enterocytes, luminal recycling may change, shifting portal delivery and altering systemic exposure. If a drug depends on hepatic uptake transporters, competition could reduce liver entry, while enhanced biliary secretion could lower plasma levels. These effects are often timing dependent, and meal context can magnify them by changing bile flow and micellar solubilization [166].

The second question is whether comedications alter phase II capacity. Inducers or inhibitors of UGT and SULT activity can shift conjugate patterns and therefore redirect which metabolites are available for enterohepatic cycling and local reactivation. Protein binding also matters because highly bound drugs can alter free fractions of silymarin species, especially in hypoalbuminemia or inflammatory states [19].

Clinically, interaction risk is highest when the co-administered drug has a narrow therapeutic window, relies on transporter-mediated clearance, or is given in patients with cholestasis. Studies should therefore report concomitant medications, dosing time, and exposure-verified metabolite panels. This framework supports rational caution and it avoids overclaiming interactions that are not supported by measured pharmacokinetics [1].

11. Minimum Reporting Standards and Falsifiable Hypotheses

Minimum reporting standards are essential for silymarin research, because many published findings cannot be compared in a meaningful way. Trials often report a dose without documenting what the product contained, what metabolites circulated, or when samples were taken, and these gaps create false disagreement across studies. If exposure is not verified, a null result may simply reflect low solubilization, poor adherence, or sampling that missed enterohepatic pulses [167].

This section proposes practical standards that make studies interpretable across laboratories and clinical cohorts. The goal is not to make every experiment expensive, it is to ensure that key variables are measured and reported consistently, so that mechanistic claims remain tethered to human-relevant chemistry.

We then translate these standards into falsifiable hypotheses and trial designs that can be tested with realistic sampling schedules. By linking metabolite-resolved exposure to window-specific biomarkers, and by stratifying participants when variability is predictable, future studies can move from suggestive associations to results that can actually guide clinical practice, Table 1.

Table 1. Minimum reporting standards that make silymarin studies comparable and mechanistically interpretable.

Domain	Minimum to Report	Practical Approach
Product identity and batch fingerprint	Plant part, extraction approach, excipients, isomer-resolved composition when feasible, chromatographic fingerprint	Provide HPLC or LC-MS profiles with reference standards and calibration ranges, report batch code and storage
Isomer resolution	Relative and absolute amounts of silybin A and B, isosilybins, silychristin, silydianin	Use chiral or high-resolution LC when available, otherwise justify limits and report surrogate ratios
Conjugate profiling	Glucuronide and sulphate panels in plasma and urine, not only aglycones or total hydrolysis	Targeted LC-MS/MS with authentic conjugates, when possible, report limits of quantification and hydrolysis conditions
Unbound exposure	Unbound fractions of key species under physiological protein conditions, with albumin levels reported	Equilibrium dialysis or validated ultrafiltration, include inflammatory or hypoalbuminemic states when relevant
Time-anchored sampling	Pre-specified sampling windows that capture absorption and recirculation, with clock time documented	At minimum 0, 15, 60, 240 min, and at least one point beyond 12 h, plus interval urine collections

Table 1. *Cont.*

Domain	Minimum to Report	Practical Approach
Surrogates for bile and lumen	Proxies that reflect bile-first routing and intestinal exposure when direct sampling is impossible	Faecal metabolite panels, bile acid timing markers, optional duodenal aspirates in clinical settings
Transport-competent models	Polarised intestinal and hepatic systems with verified vectorial transport and realistic solubilization	Gut epithelia with apical and basolateral compartments, hepatocyte sandwich cultures, bile salt micelles in dosing
Beta-glucuronidase and sulphatase awareness	Explicit design to distinguish spontaneous effects from local deconjugation-driven microbursts	Include enzyme inhibitors or knockdowns, report enzyme activity in media, avoid artefactual hydrolysis
Time-resolved endpoints	Early signalling edits and later remodelling readouts, linked to exposure windows	Collect biomarkers at early and late timepoints, align omics and proteostasis measures to kinetics
Data transparency	Key raw data and processing details that enable reanalysis and comparison across studies	Share assay protocols, QC rules, and anonymized metabolite tables, provide scripts when possible
Representative NAFLD/MASLD clinical trials	Silymarin 94 mg t.i.d. × 12 months, NASH patients (n = 179); ALT reduction −28%; AST reduction −22% [168].	Report product batch fingerprint, silybin A/B ratio, ALT/AST at baseline and ≥2 follow-up points, imaging endpoint (MRI-PDFF or controlled attenuation parameter), and meal/dosing schedule; conduct metabolite-verified adherence check at ≥1 timepoint
Representative alcoholic liver disease clinical trials	Silymarin 140 mg t.i.d. × 41 months, alcoholic cirrhosis (n = 170); 4-year survival 58% vs. 39% placebo [169]. 450 mg/day × 24 months, alcoholic cirrhosis (n = 200); no significant survival benefit; no exposure verification. Both trials lacked conjugate profiling; interindividual variation in enterohepatic cycling was not assessed [170].	Report abstinence status and alcohol biomarkers (CDT, EtG-glucuronide), serum conjugate panel at ≥2 timepoints, albumin and bilirubin (unbound fraction proxies), Child-Pugh/MELD score, and cause-specific mortality; stratify by hypoalbuminemia (albumin < 3.5 g/dL)
β-Glucuronidase initiation criteria	Deconjugation-driven aglycone regeneration is most relevant when: (1) faecal β-glucuronidase activity is elevated (>50 nmol/min/mg protein by 4-nitrophenyl glucuronide assay); (2) intestinal inflammation is present (faecal calprotectin > 50 µg/g); (3) microbial gene abundance for GUS families (loop 1, no-loop, mini-loop) is high by shotgun metagenomics; or (4) plasma aglycone-to-conjugate ratio rises unexpectedly post-dose without formulation change	Measure faecal β-glucuronidase activity at baseline; add selective inhibitors (e.g., inh-1) in parallel assay arms to attribute conjugate-vs. aglycone-driven effects; report GUS metabotype as a stratification variable; cross-validate with plasma microburst proxy (aglycone-to-total ratio at 2 h post-dose)

11.1. Exposure Standards

Exposure reporting is the main reason why silymarin studies cannot be compared with confidence, because the administered mixture is rarely the mixture that circulates. Minimum standards should therefore describe, with detail, which chemical species reached

participants, when they appeared, and what fraction of each was pharmacologically available [171].

First, authors should resolve major isomers whenever the analytical platform allows because silybin A and silybin B, and the corresponding isosilybins, can yield different metabolite patterns and transporter routing. Batch fingerprints should be provided, ideally with authentic standards and explicit calibration ranges, so that total silymarin is not a black box. Second, conjugate profiling should be routine, since plasma and urine are dominated by glucuronides and sulphates, and these metabolites define the real systemic exposure landscape [172].

Third, reporting must distinguish total concentrations from unbound concentrations, since strong protein binding can inflate totals while leaving little free material for cellular entry or membrane transport. Unbound fractions should be measured by equilibrium dialysis or validated ultrafiltration, using protein levels that match the studied population, including inflammatory states when relevant. Without unbound data, mechanistic claims about receptor engagement, intracellular redox events, or target occupancy re-main speculative [173].

Finally, sampling time must be explicit and dense enough to capture early absorption and later recirculation waves, with pre-planned windows that include 15, 60, and 240 min, and at least one late point beyond 12 h. Because bile and lumen are rarely sampled directly, studies should use practical surrogates, such as faecal metabolite profiles, bile acid-linked timing markers, or duodenal aspirates in clinical settings, and justify their choice transparently [174].

11.2. Model/Assay Standards

A credible experimental toolbox for silymarin must reproduce routing, timing, and realistic chemical speciation. Transport competent systems are essential, including polarised intestinal epithelia, hepatocyte sandwich cultures, and microphysiological gut–liver chips that preserve apical and basolateral separation. These platforms allow conjugates to be secreted directionally, and they capture bile-facing and lumen-facing enrichment that simple monolayers miss [175].

Time-stamped omics strengthens interpretation because silymarin signals are pulsed. Transcriptomics, redox proteomics, and metabolomics should be collected at early points, such as 15, 60, and 240 min, then again at 6 to 24 h, so priming is separated from remodelling. Proteostasis endpoints, including autophagy flux and lysosomal gene induction, should follow the same timeline [176].

Target engagement tools can add specificity, even when candidates are debated. CETSA and thermal proteome profiling can identify proteins whose stability shifts during exposure, and these changes can be cross-checked with unbound concentrations and conjugate panels. This approach helps avoid attributing effects to targets that are not reachable in vivo [177].

Spatial methods are especially valuable because microbursts are localized. Imaging of redox reporters, spatial transcriptomics, and MALDI imaging mass spectrometry can connect metabolites to responsive cell neighbourhoods. When these tools are combined with strong exposure verification and standardized meals and sampling, mechanistic claims become reproducible and clinically interpretable [143].

11.3. Testable Hypotheses + Trial Designs

Testable hypotheses should connect silymarin exposure patterns to measurable, time-anchored biology in humans. A practical first hypothesis is that early post-intake conjugate

dominance predicts a transient NRF2-related transcriptional shift in circulating cells and mucosal markers [178].

Trials can operationalize this by predefining window-specific biomarker panels and sampling times. In the 0 to 2 h window, measure conjugate ratios, unbound exposure, and rapid redox responses such as heme oxygenase 1 transcripts. In the 2 to 8 h window, look for secondary exposure waves alongside inflammatory editing, using cytokine kinetics and NF-kB-responsive signatures [179].

A second hypothesis is that late effects depend on microbial processing and therefore differ by metabotype. Participants can be stratified using baseline stool beta-glucuronidase activity, targeted metabolomics, or microbiome gene abundance, then randomised within strata. Late sampling at 12 to 48 h should prioritise proteostasis outputs, including autophagy flux markers and lipid handling readouts relevant to MASLD [180].

Formulation timing matching is a third hypothesis, because dissolution and micellarization interact with meals and bile flow. A crossover design comparing formulations, with fixed meal timing and the same biomarker windows, can reveal whether improved exposure routing translates into clinically meaningful endpoints. Including simple adherence checks and metabolite-based exposure verification would prevent null results caused by noncompliance or underdosing alone [69].

12. Conclusions

Silymarin, derived from Silybum marianum, emerges as a multifaceted nutraceutical with significant potential in liver disease management, particularly for metabolic-associated steatotic liver disease (MASLD) and alcohol-associated liver disease (ALD). This review reframes its activity through a spatial pharmacology and chronopharmacology lens, emphasising that its efficacy hinges not on high systemic aglycone levels but on localised, time-dependent exposure to phase II conjugates and their reactivation. By integrating pharmacokinetics, transporter topology, and signalling modules like Keap1/NRF2, NF-κB, and AMPK-mTOR-TFEB, we highlight how silymarin modulates redox signalling and proteostasis in distinct temporal windows: early (0–2 h) conjugate-dominant phase with β-glucuronidase-mediated microbursts; intermediate (2–8 h) enterohepatic recirculation pulses; and late (8–48 h) microbial catabolite contributions. These windows engage transient quinone cycling, micro-H_2O_2 relays, autophagy/mitophagy, and mitochondrial quality control, shifting cellular tone from pro-oxidant to cytoprotective without requiring sustained high concentrations.

Human pharmacokinetic data reveal that oral silymarin undergoes rapid glucuronidation and sulfation in enterocytes and hepatocytes, resulting in protein-bound conjugates that are routed via transporters (e.g., OATP, MRP2) toward bile and intestinal mucosa. This "bile-first" logic explains low plasma aglycone (<1% unbound) despite documented hepatoprotective effects. Local reactivation in inflamed microenvironments, amplified by immune cell β-glucuronidase and oxidative tone, generates brief aglycone pulses that tune signalling thresholds. For instance, NRF2 activation upregulates NQO1, HO-1, and GCLC, restoring glutathione reserves, while context-dependent NF-κB modulation reduces cytokine amplification. Proteostatic remodelling via AMPK-mTOR-TFEB coupling enhances autophagy flux, mitigating lipid peroxidation, ER stress, and mitochondrial dysfunction in MASLD/ALD models.

Clinical evidence, though inconsistent, supports this framework. Meta-analyses show silymarin reduces aminotransferases and improves histology in MASLD: 240 mg/day for 48 weeks lowered ALT by 15–20%, with better outcomes in exposure-verified trials. In ALD, it tempers oxidative injury and fibrosis, but heterogeneity arises from variable formulations, dosing (200–1000 mg/day), meal timing, and metabotypes. Responder subgroups, those

with high β-glucuronidase activity or altered bile acids, exhibit stronger NRF2 induction and inflammatory resolution. Formulation innovations (e.g., phospholipid complexes) enhance micellarization and biliary enrichment, increasing bioavailability 5–10-fold without proportional systemic rise, aligning with localised action.

A SWOT analysis underscores silymarin's strengths: pleiotropic, low-toxicity modulation of gut–liver axis, substantiated by over 5000 publications showing antioxidant, anti-inflammatory, and antifibrotic effects. It excels in hormetic regimes, where mild redox perturbations prime adaptive responses outlasting exposure. However, weaknesses include compositional variability (silybin A/B ~50%, isosilybins ~10%), underestimation of unbound/local exposure, and lack of direct microburst validation in human tissues. Traditional PK metrics fail to capture compartmentalization, leading to noisy trials where null results stem from poor standardisation rather than inefficacy.

Opportunities lie in precision approaches: advanced formulations targeting bile recirculation; metabotyping via faecal β-glucuronidase, microbiome profiling, and phenolic catabolites to identify responders; and integration with MASLD therapies (e.g., PPAR agonists, SIRT1 activators) for synergistic proteostasis support. Emerging tools like spatial metabolomics, thermal proteome profiling, and redox imaging can confirm local quinone/H_2O_2 relays and TFEB-mediated lysosomal biogenesis. In ALD, combining silymarin with abstinence programmes could enhance mitochondrial recovery, as preclinical data link NRF2 to reduced ethanol-induced ROS.

Threats include methodological inertia, trials without isomer-resolved fingerprints, time-anchored sampling, or unbound fractions perpetuate ambiguity, and safety concerns in vulnerable populations (cholestasis, hypoalbuminemia, polypharmacy). Herb–drug interactions (e.g., CYP2C9 inhibition, transporter competition) remain understudied, risking narrowed safety windows. Regulatory classification as a supplement discourages rigorous, large-scale trials, stalling translation.

In summary, silymarin's case is strongest as a spatially gated, time-dependent modulator rather than a generic antioxidant. Future studies must adopt minimum reporting standards: batch composition (HPLC-MS isomer quantification), PK profiling (conjugates, unbound fractions at 0–48 h), stratification (metabotype, inflammation, comedications), and endpoints reflecting remodelling (MRI-PDFF for steatosis, LC3/p62 for autophagy, cytokines for resolution). Falsifiable hypotheses include: (1) high β-glucuronidase metabotypes show greater NRF2 induction and ALT reduction; (2) meal-timed dosing prolongs intermediate window effects; and (3) phospholipid formulations enhance biliary microbursts without systemic toxicity. Implementing these will enable precision use, resolving heterogeneity and establishing silymarin's credible role in liver health.

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References

1. Gillessen, A.; Schmidt, H.H.J. Silymarin as supportive treatment in liver diseases: A narrative review. *Adv. Ther.* **2020**, *37*, 1279–1301. [CrossRef]
2. Tvrdý, V.; Pourová, J.; Jirkovský, E.; Křen, V.; Valentová, K.; Mladěnka, P. Systematic review of pharmacokinetics and potential pharmacokinetic interactions of flavonolignans from silymarin. *Med. Res. Rev.* **2021**, *41*, 2195–2246. [CrossRef]
3. Muchiri, R.N.; van Breemen, R.B. Chemical standardization of milk thistle (*Silybum marianum* L.) extract using UHPLC-MS/MS and the method of standard addition. *J. Am. Soc. Mass Spectrom.* **2024**, *35*, 1726–1732. [CrossRef] [PubMed]
4. Chambers, C.S.; Holečková, V.; Petrásková, L.; Biedermann, D.; Valentová, K.; Buchta, M.; Křen, V. The silymarin composition. . . and why does it matter??? *Food Res. Int.* **2017**, *100*, 339–353. [CrossRef] [PubMed]
5. Kudláčková, B.; Misák, P.; Pluháčková, H. Silymarin and fatty acid profiles of milk thistle (*Silybum marianum* L.) genotypes. *Plant Foods Hum. Nutr.* **2025**, *80*, 158. [CrossRef]
6. Petrásková, L.; Káňová, K.; Biedermann, D.; Křen, V.; Valentová, K. Simple and rapid HPLC separation and quantification of flavonoid, flavonolignans, and 2,3-dehydroflavonolignans in silymarin. *Foods* **2020**, *9*, 116. [CrossRef] [PubMed]
7. Vajdle, O.; Jurčacková, Z.; Sůkupová, S.; Hemšek, E.; Biedermann, D.; Valentová, K.; Křen, V. Determination of flavonolignan compositional ratios in *Silybum marianum* (milk thistle) extracts using high-performance liquid chromatography. *Molecules* **2024**, *29*, 2949. [CrossRef] [PubMed]
8. Křen, V. Chirality Matters: Biological Activity of Optically Pure Silybin and Its Congeners. *Int. J. Mol. Sci.* **2021**, *22*, 7885. [CrossRef]
9. Jančová, P.; Šiller, M.; Anzenbacherová, E.; Křen, V.; Anzenbacher, P.; Šimánek, V. Evidence for differences in regioselective and stereoselective glucuronidation of silybin diastereomers from milk thistle (Silybum marianum) by human UDP-glucuronosyltransferases. *Xenobiotica* **2011**, *41*, 743–751. [CrossRef]
10. Křen, V.; Valentová, K. Silybin and its congeners: From traditional medicine to molecular effects. *Nat. Prod. Rep.* **2022**, *39*, 1264–1281. [CrossRef]
11. Csupor, D.; Csorba, A.; Hohmann, J. Recent advances in the analysis of flavonolignans of Silybum marianum. *J. Pharm. Biomed. Anal.* **2016**, *130*, 301–317. [CrossRef] [PubMed]
12. Di Costanzo, A.; Angelico, R. Formulation strategies for enhancing the bioavailability of silymarin: The state of the art. *Molecules* **2019**, *24*, 2155. [CrossRef]
13. Porter, C.J.H.; Trevaskis, N.L.; Charman, W.N. Lipids and lipid-based formulations: Optimising the oral delivery of lipophilic drugs. *Nat. Rev. Drug Discov.* **2007**, *6*, 231–248. [CrossRef]
14. Yamasaki, K.; Sato, H.; Minagoshi, S.; Kyubun, K.; Anraku, M.; Miyamura, S.; Watanabe, H.; Taguchi, K.; Seo, H.; Maruyama, T.; et al. The binding of silibinin, the main constituent of silymarin, to site I on human serum albumin. *Biol. Pharm. Bull.* **2017**, *40*, 310–317. [CrossRef]
15. Wen, Z.; Dumas, T.E.; Schrieber, S.J.; Hawke, R.L.; Fried, M.W.; Smith, P.C. Pharmacokinetics and metabolic profile of free, conjugated, and total silymarin flavonolignans in human plasma after oral administration of milk thistle extract. *Drug Metab. Dispos.* **2008**, *36*, 65–72. [CrossRef]
16. Xie, Y.; Zhang, D.; Zhang, J.; Yuan, J. Metabolism, transport and drug-drug interactions of silymarin. *Molecules* **2019**, *24*, 3693. [CrossRef] [PubMed]
17. Miranda, S.R.; Lee, J.K.; Brouwer, K.L.; Wen, Z.; Smith, P.C.; Hawke, R.L. Hepatic metabolism and biliary excretion of silymarin flavonolignans in isolated perfused rat livers: Role of multidrug resistance-associated protein 2 (Abcc2). *Drug Metab. Dispos.* **2008**, *36*, 2219–2226. [CrossRef]
18. Pferschy-Wenzig, E.-M.; Kunert, O.; Thumann, T.; Moissl-Eichinger, C.; Bauer, R. Characterization of metabolites from milk thistle flavonolignans generated by human fecal microbiota. *Phytochemistry* **2023**, *215*, 113834. [CrossRef]
19. Xie, Y.; Miranda, S.R.; Hoskins, J.M.; Hawke, R.L. Role of UDP-glucuronosyltransferase 1A1 in the metabolism and pharmacokinetics of silymarin flavonolignans in patients with HCV and NAFLD. *Molecules* **2017**, *22*, 142. [CrossRef] [PubMed]
20. Tomisova, K.; Jarosova, V.; Marsik, P.; Mascellani Bergo, A.; Cinek, O.; Hlinakova, L.; Kloucek, P.; Janousek, V.; Valentová, K.; Havlik, J. Mutual interactions of silymarin and colon microbiota in healthy young and healthy elder subjects. *Mol. Nutr. Food Res.* **2024**, *68*, e2400500. [CrossRef] [PubMed]

21. Dashnyam, P.; Mudunuddla, R.; Hsieh, T.-J.; Lin, T.-C.; Lin, H.-Y.; Chen, P.-Y.; Hsu, C.-Y.; Lin, C.-H. β-Glucuronidases of opportunistic bacteria are the major contributors to xenobiotic-induced toxicity in the gut. *Sci. Rep.* **2018**, *8*, 16372. [CrossRef]
22. Valentová, K.; Havlík, J.; Kosina, P.; Papoušková, B.; Jaimes, J.D.; Káňová, K.; Petrásková, L.; Ulrichová, J.; Křen, V. Biotransformation of silymarin flavonolignans by human fecal microbiota. *Metabolites* **2020**, *10*, 29. [CrossRef]
23. Meroño, T.; Peron, G.; Gargari, G.; González-Domínguez, R.; Miñarro, A.; Vegas-Lozano, E.; Hidalgo-Liberona, N.; Del Bo, C.; Bernardi, S.; Kroon, P.A.; et al. The relevance of urolithins-based metabotyping for assessing the effects of a polyphenol-rich dietary intervention on intestinal permeability: A post-hoc analysis of the MaPLE trial. *Food Res. Int.* **2022**, *159*, 111632. [CrossRef] [PubMed]
24. Sun, Y.; Xie, L.; Zhang, J.; Liu, R.; Li, H.; Yang, Y.; Wu, Y.; Peng, Y.; Wang, G.; Hughes-Medlicott, N.; et al. Intestine versus liver? Uncovering the hidden major metabolic organs of silybin in rats. *Drug Metab. Dispos.* **2025**, *53*, 100005. [CrossRef] [PubMed]
25. Chang, C.; Sosa, A.P.; González, A.; Qian, Y.; Rupasinghe, H.P.V.; Hajeer, A.H. Novel micellar formulation of silymarin (milk thistle) with enhanced bioavailability in a double-blind, randomized, crossover human trial. *Pharmaceutics* **2025**, *17*, 880. [CrossRef]
26. Bi, X.; Yuan, Z.; Qu, B.; Zhou, H.; Liu, Z.; Xie, Y. Piperine enhances the bioavailability of silybin via inhibition of efflux transporters BCRP and MRP2. *Phytomedicine* **2019**, *54*, 98–108. [CrossRef]
27. Lynch, K.D.; Montonye, M.L.; Tian, D.D.; Arman, T.; Oyanna, V.O.; Bechtold, B.J.; Graf, T.N.; Oberlies, N.H.; Paine, M.F.; Clarke, J.D. Hepatic organic anion transporting polypeptides mediate disposition of milk thistle flavonolignans and pharmacokinetic silymarin-drug interactions. *Phytother. Res.* **2021**, *35*, 3286–3297. [CrossRef]
28. Wu, K.-C.; Lin, C.-J. The regulation of drug-metabolizing enzymes and membrane transporters by inflammation: Evidences in inflammatory diseases and age-related disorders. *J. Food Drug Anal.* **2019**, *27*, 48–59. [CrossRef] [PubMed]
29. Vrba, J.; Papoušková, B.; Kosina, P.; Lněničková, K.; Valentová, K.; Ulrichová, J. Identification of human sulfotransferases active towards silymarin flavonolignans and taxifolin. *Metabolites* **2020**, *10*, 329. [CrossRef]
30. Tayyab, S.; Feroz, S.R. Serum albumin: Clinical significance of drug binding and development as drug delivery vehicle. *Adv. Protein Chem. Struct. Biol.* **2021**, *123*, 193–218. [CrossRef]
31. Bohnert, T.; Gan, L.-S. Plasma protein binding: From discovery to development. *J. Pharm. Sci.* **2013**, *102*, 2953–2994. [CrossRef]
32. Toma, C.-M.; Imre, S.; Vari, C.-E.; Muntean, D.-L.; Tero-Vescan, A. Ultrafiltration method for plasma protein binding studies and its limitations. *Processes* **2021**, *9*, 382. [CrossRef]
33. Murtaza, G.; Ullah, N.; Mukhtar, F.; Nawazish, S.; Muneer, S.; Mariam. Phytotherapeutics: The Emerging Role of Intestinal and Hepatocellular Transporters in Drug Interactions with Botanical Supplements. *Molecules* **2017**, *22*, 1699. [CrossRef]
34. Yuan, Z.W.; Li, Y.Z.; Liu, Z.Q.; Feng, S.L.; Zhou, H.; Liu, C.X.; Liu, L.; Xie, Y. Role of tangeretin as a potential bioavailability enhancer for silybin: Pharmacokinetic and pharmacological evaluation. *Pharmacol. Res.* **2018**, *128*, 153–166. [CrossRef] [PubMed]
35. Xu, P.; Zhou, H.; Li, Y.Z.; Yuan, Z.W.; Liu, C.X.; Liu, L.; Xie, Y. Baicalein enhances the oral bioavailability and hepatoprotective effects of silybin through the inhibition of efflux transporters BCRP and MRP2. *Front. Pharmacol.* **2018**, *9*, 1115. [CrossRef] [PubMed]
36. Bechtold, B.J.; Lynch, K.D.; Oyanna, V.O.; Call, M.R.; Graf, T.N.; Oberlies, N.H.; Clarke, J.D. Rifampin- and silymarin-mediated pharmacokinetic interactions of exogenous and endogenous substrates in a transgenic OATP1B mouse model. *Mol. Pharm.* **2024**, *21*, 2284–2297. [CrossRef]
37. 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. *Evid.-Based Complement. Altern. Med.* **2018**, *2018*, 1507834. [CrossRef] [PubMed]
38. Naumann, S.; Haller, D.; Eisner, P.; Schweiggert-Weisz, U. Mechanisms of interactions between bile acids and plant compounds-A review. *Int. J. Mol. Sci.* **2020**, *21*, 6495. [CrossRef]
39. Ibarra, M.; Trocóniz, I.F.; Fagiolino, P. Enteric reabsorption processes and their impact on drug pharmacokinetics. *Sci. Rep.* **2021**, *11*, 5794. [CrossRef] [PubMed]
40. Petrásková, L.; Káňová, K.; Biedermann, D.; Valentová, K.; Křen, V. Metabolic profiling of silymarin constituents in urine and feces of healthy volunteers: A 90-day study. *J. Funct. Foods* **2023**, *100*, 105374. [CrossRef]
41. Żyżyńska-Granica, B.; Gierlikowska, B.; Parzonko, A.; Kiss, A.K.; Granica, S. The bioactivity of flavonoid glucuronides and free aglycones in the context of their absorption, II phase metabolism and deconjugation at the inflammation site. *Food Chem. Toxicol.* **2020**, *135*, 110929. [CrossRef]
42. Awolade, P.; Cele, N.; Kerru, N.; Gummidi, L.; Oluwakemi, E.; Singh, P. Therapeutic significance of β-glucuronidase activity and its inhibitors: A review. *Eur. J. Med. Chem.* **2019**, *187*, 111921. [CrossRef]
43. Ervin, S.M.; Li, H.; Lim, L.; Roberts, L.R.; Liang, X.; Mani, S.; Redinbo, M.R. Gut microbial β-glucuronidases reactivate estrogens as components of the estrobolome that reactivate estrogens. *J. Biol. Chem.* **2019**, *294*, 18586–18599. [CrossRef]
44. Shi, Q.; Haenen, G.R.; Maas, L.; Arlt, V.M.; Spina, D.; Riffo Vasquez, Y.; Moonen, E.; Veith, C.; Van Schooten, F.J.; Godschalk, R.W.L. Inflammation-associated extracellular β-glucuronidase alters cellular responses to the chemical carcinogen benzo[a]pyrene. *Arch. Toxicol.* **2016**, *90*, 2261–2273. [CrossRef] [PubMed]

45. Candeliere, F.; Raimondi, S.; Ranieri, R.; Musmeci, E.; Zambon, A.; Amaretti, A.; Rossi, M. β-Glucuronidase pattern predicted from gut metagenomes indicates potentially diversified pharmacomicrobiomics. *Front. Microbiol.* **2022**, *13*, 826994. [CrossRef] [PubMed]
46. Espín, J.C.; González-Sarrías, A.; Tomás-Barberán, F.A. The gut microbiota: A key factor in the therapeutic effects of (poly)phenols. *Biochem. Pharmacol.* **2017**, *139*, 82–93. [CrossRef] [PubMed]
47. Elmassry, M.M.; Kim, S.; Busby, B. Predicting drug-metagenome interactions: Variation in the microbial β-glucuronidase level in the human gut metagenomes. *PLoS ONE* **2021**, *16*, e0244876. [CrossRef] [PubMed]
48. Lock, J.Y.; Carlson, T.L.; Carrier, R.L. Mucus models to evaluate the diffusion of drugs and particles. *Adv. Drug Deliv. Rev.* **2018**, *124*, 34–49. [CrossRef]
49. Dinkova-Kostova, A.T.; Kostov, R.V.; Canning, P. Keap1, the cysteine-based mammalian intracellular sensor for electrophiles and oxidants. *Arch. Biochem. Biophys.* **2017**, *617*, 84–93. [CrossRef]
50. Margaritelis, N.V.; Cobley, J.N.; Paschalis, V.; Veskoukis, A.S.; Theodorou, A.A.; Kyparos, A.; Nikolaidis, M.G. In vivo imaging with genetically encoded redox biosensors. *Int. J. Mol. Sci.* **2020**, *21*, 8164. [CrossRef] [PubMed]
51. Speisky, H.; Shahidi, F.; Costa de Camargo, A.; Fuentes, J. Revisiting the oxidation of flavonoids: Loss, conservation or enhancement of their antioxidant properties. *Antioxidants* **2022**, *11*, 133. [CrossRef]
52. Lee, W.-S.; Ham, W.; Kim, J. Roles of NAD(P)H:quinone Oxidoreductase 1 in Diverse Diseases. *Life* **2021**, *11*, 1301. [CrossRef]
53. Santacroce, G.; Gentile, A.; Soriano, S.; Novelli, A.; Lenti, M.V.; Di Sabatino, A. Glutathione: Pharmacological aspects and implications for clinical use in non-alcoholic fatty liver disease. *Front. Med.* **2023**, *10*, 1124275. [CrossRef]
54. Parvez, S.; Long, M.J.C.; Poganik, J.R. Redox signaling by reactive electrophiles and oxidants. *Chem. Rev.* **2018**, *118*, 8798–8888. [CrossRef]
55. Sies, H. Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biol.* **2017**, *11*, 613–619. [CrossRef] [PubMed]
56. Varadaraj, K.; Kumari, S.S. Lens aquaporins function as peroxiporins to facilitate membrane transport of hydrogen peroxide. *Biochem. Biophys. Res. Commun.* **2020**, *524*, 1025–1029. [CrossRef] [PubMed]
57. Thiagarajah, J.R.; Chang, J.; Goettel, J.A.; Verkman, A.S.; Lencer, W.I. Aquaporin-3 mediates hydrogen peroxide-dependent responses to environmental stress in colonic epithelia. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 568–573. [CrossRef] [PubMed]
58. da Silva, I.V.; Soveral, G. Aquaporins in immune cells and inflammation: New targets for drug development. *Int. J. Mol. Sci.* **2021**, *22*, 1845. [CrossRef]
59. Rhee, S.G.; Woo, H.A.; Kang, D. The role of peroxiredoxins in the transduction of H_2O_2 signals. *Antioxid. Redox Signal.* **2018**, *28*, 537–557. [CrossRef]
60. Pak, V.V.; Ezeriņa, D.; Lyublinskaya, O.G.; Pedre, B.; Tyurin-Kuzmin, P.A.; Mishina, N.M.; Thauvin, M.; Young, D.; Wahni, K.; Martínez Gache, S.A.; et al. Ultrasensitive genetically encoded indicator for hydrogen peroxide identifies roles for the oxidant in cell migration and mitochondrial function. *Cell Metab.* **2020**, *31*, 642–653.e6. [CrossRef]
61. Calabrese, E.J.; Kozumbo, W.J. The hormetic dose-response mechanism: Nrf2 activation. *Pharmacol. Res.* **2021**, *167*, 105526. [CrossRef]
62. Muri, J.; Wolleb, H.; Broz, P.; Carreira, E.M.; Kopf, M. Electrophilic Nrf2 activators and itaconate inhibit inflammation at low dose and promote IL-1β production and inflammatory apoptosis at high dose. *Redox Biol.* **2020**, *36*, 101647. [CrossRef]
63. Armani, S.; Sottani, C.; Cariani, L. Effect of changes in metabolic enzymes and transporters on drug metabolism in the context of liver disease: Impact on pharmacokinetics and drug-drug interactions. *Br. J. Clin. Pharmacol.* **2024**, *90*, 942–958. [CrossRef] [PubMed]
64. Lv, H.; Yang, H.; Wang, Z.; Zhang, S.; Yang, J.; Kan, Q.; Zhang, J.; Li, Y.; Zhang, L.; Zhou, W. Nrf2 signaling and autophagy are complementary in protecting lipopolysaccharide/d-galactosamine-induced acute liver injury by licochalcone A. *Cell Death Dis.* **2019**, *10*, 313. [CrossRef] [PubMed]
65. Villegas-Aguilar, M.D.C.; Cádiz-Gurrea, M.D.L.; Herranz-López, M.; Barrajón-Catalán, E.; Arráez-Román, D.; Fernández-Ochoa, Á.; Segura-Carretero, A. An untargeted metabolomics approach applied to the study of the bioavailability and metabolism of three different bioactive plant extracts in human blood samples. *Food Funct.* **2024**, *15*, 9176–9190. [CrossRef] [PubMed]
66. Lazzeroni, M.; Petrangolini, G.; Legarreta Iriberri, J.A.; Pascual Avellana, J.; Tost Robusté, D.; Cagnacci, S.; Macis, D.; Aristarco, V.; Bonanni, B.; Morazzoni, P.; et al. Development of an HPLC-MS/MS method for the determination of silybin in human plasma, urine and breast tissue. *Molecules* **2020**, *25*, 2918. [CrossRef]
67. Morgenstern, C.; Lastres-Becker, I.; Demirdöğen, B.C.; Costa, V.M.; Daiber, A.; Foresti, R.; Mann, G.E.; Cuadrado, A.; Copple, I.M. Biomarkers of NRF2 signalling: Current status and future challenges. *Redox Biol.* **2024**, *72*, 103134. [CrossRef]
68. Klionsky, D.J.; Abdel-Aziz, A.K.; Abdelfatah, S.; Abdellatif, M.; Abdoli, A.; Abel, S.; Abeliovich, H.; Abildgaard, M.H.; Abudu, Y.P.; Acevedo-Arozena, A.; et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition). *Autophagy* **2021**, *17*, 1–382. [CrossRef]

69. Méndez-Sánchez, N.; Dibildox-Martinez, M.; Sosa-Noguera, J.; Sánchez-Medal, R.; Flores-Murrieta, F.J. Superior silybin bioavailability of silybin-phosphatidylcholine complex in oily-medium soft-gel capsules versus conventional silymarin tablets in healthy volunteers. *BMC Pharmacol. Toxicol.* **2019**, *20*, 5. [CrossRef]
70. Vargas-Mendoza, N.; Morales-González, Á.; Morales-Martínez, M.; Soriano-Ursúa, M.A.; Delgado-Olivares, L.; Sandoval-Gallegos, E.M.; Madrigal-Bujaidar, E.; Álvarez-González, I.; Madrigal-Santillán, E.; Morales-Gonzalez, J.A. Flavolignans from silymarin as Nrf2 bioactivators and their therapeutic applications. *Biomedicines* **2020**, *8*, 122. [CrossRef]
71. Vrba, J.; Kosina, P.; Ulrichová, J.; Dvořák, Z. Metabolism of flavonolignans in human hepatocytes. *J. Pharm. Biomed. Anal.* **2018**, *152*, 94–101. [CrossRef]
72. Pellock, S.J.; Redinbo, M.R. Glucuronides in the gut: Sugar-driven symbioses between microbe and host. *J. Biol. Chem.* **2017**, *292*, 8569–8576. [CrossRef]
73. Tebay, L.E.; Robertson, H.; Durant, S.T.; Vitale, S.R.; Penning, T.M.; Dinkova-Kostova, A.T.; Hayes, J.D. Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. *Free Radic. Biol. Med.* **2015**, *88*, 108–146. [CrossRef] [PubMed]
74. Hayes, J.D.; Dinkova-Kostova, A.T.; Tew, K.D. Oxidative stress in cancer. *Cancer Cell* **2020**, *38*, 167–197. [CrossRef]
75. Zeng, M.; Sun, R.; Basu, S.; Ma, Y.; Ge, S.; Yin, T.; Gao, S.; Zhang, J.; Hu, M. Disposition of flavonoids via recycling: Direct biliary excretion of enterically or extrahepatically derived flavonoid glucuronides. *Mol. Nutr. Food Res.* **2016**, *60*, 1006–1019. [CrossRef]
76. Sharma, P.; Asediya, V.; Kalra, G.; Sultana, S.; Purohit, N.; Kibitlewska, K.; Kozera, W.; Kibitlewska, M. Hepatoprotective effect of silymarin herb in prevention of liver dysfunction using pig as animal model. *Nutrients* **2025**, *17*, 3278. [CrossRef]
77. Shangguan, M.; Qi, J.; Lu, Y.; Wu, W. Comparison of the oral bioavailability of silymarin-loaded lipid nanoparticles with their artificial lipolysate counterparts: Implications on the contribution of integral structure. *Int. J. Pharm.* **2015**, *489*, 195–202. [CrossRef]
78. Krajka-Kuźniak, V.; Baer-Dubowska, W. Modulation of Nrf2 and NF-κB signaling pathways by naturally occurring compounds in relation to cancer prevention and therapy. Are combinations better than single compounds? *Int. J. Mol. Sci.* **2021**, *22*, 8223. [CrossRef]
79. Chen, C.-H.; Chang, C.-C.; Shih, T.-H.; Aljuffali, I.A.; Yeh, T.-S.; Fang, J.-Y. Self-nanoemulsifying drug delivery systems ameliorate the oral delivery of silymarin in rats with Roux-en-Y gastric bypass surgery. *Int. J. Nanomed.* **2015**, *10*, 2403–2416. [CrossRef]
80. Carregosa, D.; Pinto, C.; Ávila-Gálvez, M.A.; Bastos, P.; González-Sarrías, A.; Santos, C.N.; Mena, P. A look beyond dietary (poly)phenols: The low molecular weight phenolic metabolites and their concentrations in human circulation. *Compr. Rev. Food Sci. Food Saf.* **2022**, *21*, 3931–3962. [CrossRef] [PubMed]
81. Mokhtari-Andani, F.; Talebi-Garakani, E.; Nasiri, K.; Akbari, A. Exercise training and Silymarin consumption can ameliorate mitophagy signaling flux in hepatocytes of rats with dexamethasone-induced non-alcoholic fatty liver disease. *Sci. Rep.* **2025**, *15*, 36637. [CrossRef]
82. Hu, J.; Mesnage, R.; Tuohy, K.; Heiss, C.; Rodriguez-Mateos, A. (Poly)phenol-related gut metabotypes and human health: An update. *Food Funct.* **2024**, *15*, 2814–2835. [CrossRef]
83. Crozier, A.; Del Rio, D.; Clifford, M.N. Gold standards for realistic (poly)phenol research. *J. Agric. Food Chem.* **2018**, *66*, 8221–8223. [CrossRef] [PubMed]
84. Yang, Y.; Zhang, J. Bile acid metabolism and circadian rhythms. *Am. J. Physiol.-Gastrointest. Liver Physiol.* **2020**, *319*, G549–G563. [CrossRef]
85. Xu, H.; Chen, M.; Yu, F.; Zhang, T.; Wu, B. Circadian clock component Rev-erb α regulates diurnal rhythm of UDP-glucuronosyltransferase 1a9 and drug glucuronidation in mice. *Drug Metab. Dispos.* **2020**, *48*, 681–689. [CrossRef] [PubMed]
86. Thaiss, C.A.; Levy, M.; Korem, T.; Dohnalová, L.; Shapiro, H.; Jaitin, D.A.; David, E.; Winter, D.R.; Gury-BenAri, M.; Tatirovsky, E.; et al. Microbiota diurnal rhythmicity programs host transcriptome oscillations. *Cell* **2016**, *167*, 1495–1510.e12. [CrossRef] [PubMed]
87. Ayyar, V.S.; Sukumaran, S. Circadian rhythms: Influence on physiology, pharmacology, and therapeutic interventions. *J. Pharmacokinet. Pharmacodyn.* **2021**, *48*, 321–347. [CrossRef]
88. Koziolek, M.; Alcaro, S.; Augustijns, P.; Basit, A.W.; Grimm, M.; Hens, B.; Hoad, C.L.; Jedamzik, P.; Madla, C.M.; Maliepaard, M.; et al. The mechanisms of pharmacokinetic food-drug interactions—A perspective from the UNGAP group. *Eur. J. Pharm. Sci.* **2019**, *130*, 31–59. [CrossRef]
89. Dong, D.; Yang, D.; Lin, L.; Wang, S.; Wu, B. Circadian rhythm in pharmacokinetics and its relevance to chronotherapy. *Biochem. Pharmacol.* **2020**, *178*, 114045. [CrossRef]
90. Chi, C.; Zhang, C.; Liu, Y.; Li, B.; Wang, F.; Wang, Y. Phytosome-nanosuspensions for silybin-phospholipid complex with increased bioavailability and hepatoprotection efficacy. *Eur. J. Pharm. Sci.* **2020**, *144*, 105212. [CrossRef]
91. Sprouse, A.A.; van Breemen, R.B. Pharmacokinetic interactions between drugs and botanical dietary supplements. *Drug Metab. Dispos.* **2016**, *44*, 162–171. [CrossRef]
92. Sathe, A.G.; Brundage, R.C.; Ivaturi, V.; Cloyd, J.C.; Kapur, J. A pharmacokinetic simulation study to assess the performance of a sparse blood sampling approach to quantify early drug exposure. *Clin. Transl. Sci.* **2021**, *14*, 1444–1451. [CrossRef] [PubMed]

93. Chen, W.; Ruan, Z.; Chen, J.; Yang, D.; Zou, J.; Wang, G. Population pharmacokinetics and enterohepatic recirculation of hyzetimibe and its main metabolite in Chinese healthy subjects. *Br. J. Clin. Pharmacol.* **2022**, *88*, 3153–3161. [CrossRef]
94. Hamatani, T.; Shibue, Y.; Sawada, N.; Takagaki, T.; Hashimoto, M.; Nakada, Y.; Kakuyama, H. Pharmacokinetics, safety and metabolite profiling of minesapride, a novel 5-HT4 receptor partial agonist, in healthy elderly and young subjects. *Drug Metab. Pharmacokinet.* **2020**, *35*, 563–570. [CrossRef]
95. Enghusen-Poulsen, P.; Brander, T.; Weimann, A.; Poulsen, H.E. Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. *Nat. Metab.* **2022**, *4*, 651–662. [CrossRef]
96. Baird, L.; Yamamoto, M. The molecular mechanisms regulating the KEAP1-NRF2 pathway. *Mol. Cell. Biol.* **2020**, *40*, e00099-20. [CrossRef]
97. Suzuki, T.; Muramatsu, A.; Saito, R.; Iso, T.; Shibata, T.; Kuwata, K.; Kawaguchi, S.; Iwawaki, T.; Adachi, S.; Suda, H.; et al. Molecular mechanism of cellular oxidative stress sensing by Keap1. *Cell Rep.* **2019**, *28*, 746–758.e4. [CrossRef]
98. Yamamoto, M.; Kensler, T.W.; Motohashi, H. The KEAP1-NRF2 system: A thiol-based sensor-effector apparatus for maintaining redox homeostasis. *Physiol. Rev.* **2018**, *98*, 1169–1203. [CrossRef] [PubMed]
99. Carrasco-Wong, I.; Längst, G.; Sobrevia, L.; Casanello, P. Nrf2 pre-recruitment at Enhancer 2 is a hallmark of H_2O_2-induced epigenetic transcriptional memory in the HMOX1 gene in human umbilical artery endothelial cells. *J. Cell. Physiol.* **2024**, *239*, e31243. [CrossRef]
100. Boas, S.M.; Joyce, K.L.; Cowell, R.M. The NRF2-dependent transcriptional regulation of antioxidant defense pathways: Relevance for cell type-specific vulnerability to neurodegeneration and therapeutic intervention. *Antioxidants* **2022**, *11*, 8. [CrossRef] [PubMed]
101. Dorrington, M.G.; Fraser, I.D.C. NF-κB signaling in macrophages: Dynamics, crosstalk, and signal integration. *Front. Immunol.* **2019**, *10*, 705. [CrossRef]
102. MacDonald-Ramos, K.; Michán, L.; Martínez-Ibarra, A.; Cerbón, M. Silymarin is an ally against insulin resistance: A review. *Ann. Hepatol.* **2021**, *23*, 100255. [CrossRef]
103. Kizilirmak, C.; Bianchi, M.E.; Zambrano, S. Insights on the NF-κB system using live cell imaging: Recent developments and future perspectives. *Front. Immunol.* **2022**, *13*, 886127. [CrossRef] [PubMed]
104. Kumagai, Y.; Abiko, Y. Environmental electrophiles: Protein adducts, modulation of redox signaling, and interaction with persulfides/polysulfides. *Chem. Res. Toxicol.* **2017**, *30*, 203–219. [CrossRef] [PubMed]
105. Wang, A.G.; Son, M.; Kenna, E.; Thom, N.; Tay, S. NF-κB memory coordinates transcriptional responses to dynamic inflammatory stimuli. *Cell Rep.* **2022**, *40*, 111159. [CrossRef] [PubMed]
106. Malik, N.; Ferreira, B.I.; Hollstein, P.E.; Curtis, S.D.; Trefts, E.; Weiser Novak, S.; Yu, J.; Gilson, R.; Hellberg, K.; Fang, L.; et al. Induction of lysosomal and mitochondrial biogenesis by AMPK phosphorylation of FNIP1. *Science* **2023**, *380*, eabj5559. [CrossRef]
107. Zhang, H.; Qiu, J.; Ye, C.; Yang, R.; Li, M.; Li, Y.; Zhang, X.; Yin, L.; Shu, X. Dynamic MTORC1-TFEB feedback signaling regulates hepatic autophagy, steatosis and liver injury in long-term nutrient oversupply. *Autophagy* **2018**, *14*, 1559–1575. [CrossRef] [PubMed]
108. Youhanna, S.; Kemas, A.M.; Preiss, L.; Zhou, Y.; Shen, J.X.; Cakal, S.D.; Paqualini, F.S.; Goparaju, S.K.; Shafagh, R.Z.; Lind, J.U.; et al. Organotypic and microphysiological human tissue models for drug discovery and development-Current state-of-the-art and future perspectives. *Pharmacol. Rev.* **2022**, *74*, 141–206. [CrossRef]
109. Vega-Rubín-de-Celis, S.; Peña-Llopis, S.; Konda, M.; Brugarolas, J. Multistep regulation of TFEB by MTORC1. *Autophagy* **2017**, *13*, 464–472. [CrossRef]
110. Fougerat, A.; Montagner, A.; Loiseau, N.; Guillou, H.; Wahli, W. Peroxisome proliferator-activated receptors and their novel ligands as candidates for the treatment of non-alcoholic fatty liver disease. *Cells* **2020**, *9*, 1638. [CrossRef]
111. Zhang, Z.; Meng, Y.; Wang, Z.; Mei, Y.; Gao, S.; Wu, Y.; Du, S. Discovery of potent glucokinase and PPARγ dual-target agonists through an innovative scheme for regioselective modification of silybin. *ACS Omega* **2022**, *7*, 3812–3822. [CrossRef] [PubMed]
112. Oh, H.Y.P.; Visvalingam, V.; Wahli, W. The PPAR-microbiota-metabolic organ trilogy to fine-tune physiology. *FASEB J.* **2019**, *33*, 9706–9730. [CrossRef]
113. Pan, J.; Zhou, W.; Xu, R.; Xing, L.; Ji, G.; Dang, Y. Natural PPARs agonists for the treatment of nonalcoholic fatty liver disease. *Biomed. Pharmacother.* **2022**, *151*, 113127. [CrossRef] [PubMed]
114. Santos, L.; Benitez-Rosendo, A.; Bresque, M.; Camacho-Pereira, J.; Escande, C.; Valdez, L.B. Sirtuins: The NAD+-dependent multifaceted modulators of inflammation. *Antioxid. Redox Signal.* **2023**, *39*, 1185–1208. [CrossRef] [PubMed]
115. Della Torre, S.; Dell'Omo, G.; Dellavedova, J.; Palazzolo, L.; Scanziani, E.; Eberini, I.; Pinto, A.; Mitro, N.; Conti, P.; Villa, A.; et al. Discovery and characterization of a new class of NAD+-independent SIRT1 activators. *Pharmacol. Res.* **2024**, *204*, 107296. [CrossRef]
116. Rodriguez-Colman, M.J.; Dansen, T.B.; Burgering, B.M.T. FOXO transcription factors as mediators of stress adaptation. *Nat. Rev. Mol. Cell Biol.* **2024**, *25*, 46–64. [CrossRef]

117. Giner, M.P.; Christen, S.; Bartova, S.; Makarov, M.V.; Migaud, M.E.; Canto, C.; Moco, S. A method to monitor the NAD+ metabolome-From mechanistic to clinical applications. *Int. J. Mol. Sci.* **2021**, *22*, 10598. [CrossRef]
118. He, X.; Li, X.; Tian, W.; Li, C.; Li, P.; Zhao, J.; Yang, S.; Li, S. The role of redox-mediated lysosomal dysfunction and therapeutic strategies. *Biomed. Pharmacother.* **2023**, *165*, 115121. [CrossRef]
119. Tan, A.; Prasad, V. Past, present, and future perspectives of transcription factor EB (TFEB): Mechanisms of regulation and association with disease. *Cell Death Differ.* **2022**, *29*, 1439–1450. [CrossRef]
120. Paquette, M.; El-Houjeiri, L.; Zirden, L.C.; Puustinen, P.; Blanchette, P.; Jeong, H.; Dejgaard, K.; Siegel, P.M.; Pause, A. AMPK-dependent phosphorylation is required for transcriptional activation of TFEB and TFE3. *Autophagy* **2021**, *17*, 2637–2656. [CrossRef]
121. Settembre, C.; Di Malta, C.; Polito, V.A.; Garcia Arencibia, M.; Vetrini, F.; Erdin, S.; Erdin, S.U.; Huynh, T.; Medina, D.; Colella, P.; et al. TFEB links autophagy to lysosomal biogenesis. *Science* **2011**, *332*, 1429–1433. [CrossRef]
122. Barral, D.C.; Seabra, M.C.; Staiano, L. Current methods to analyze lysosome morphology, positioning, motility and function. *Traffic* **2022**, *23*, 238–269. [CrossRef]
123. Landsiedel, R.; Birk, B.; Demuth, P.; Fabian, E.; Hewitt, N.J.; Hollnagel, H.M.; Scheel, J. The use of toxicokinetic information for setting concentrations of in vitro toxicity tests and for interpreting their results: A proposed workflow. *Appl. Vitr. Toxicol.* **2024**, *10*, 15–26. [CrossRef]
124. Hazari, Y.; Bravo-San Pedro, J.M.; Hetz, C.; Galluzzi, L.; Kroemer, G. Autophagy in hepatic adaptation to stress. *J. Hepatol.* **2020**, *72*, 183–196. [CrossRef] [PubMed]
125. Redmann, M.; Benavides, G.A.; Berryhill, T.F.; Wani, W.Y.; Ouyang, X.; Johnson, M.S.; Darley-Usmar, V.M.; Barnes, S.; Darley-Usmar, K.M.; Zhang, J. Inhibition of autophagy with bafilomycin and chloroquine decreases mitochondrial quality and bioenergetic function in primary neurons. *Redox Biol.* **2017**, *11*, 73–81. [CrossRef]
126. Kirkin, V.; Rogov, V.V. A diversity of selective autophagy receptors determines the specificity of the autophagy pathway. *Mol. Cell* **2019**, *76*, 268–285. [CrossRef] [PubMed]
127. García-Villalba, R.; Beltrán, D.; Espín, J.C.; Selma, M.V.; Tomás-Barberán, F.A. Time course production of urolithins from ellagic acid by human gut microbiota. *J. Agric. Food Chem.* **2013**, *61*, 8797–8806. [CrossRef]
128. Huang, Y.; Xia, X.; Xu, J.; Wang, Z.; You, Y.; Du, Q. Mitophagy as a pivotal axis in non-alcoholic fatty liver disease: From pathogenic mechanisms to therapeutic strategies (Review). *Mol. Med. Rep.* **2025**, *32*, 299. [CrossRef] [PubMed]
129. Cui, Z.; Napolitano, G.; de Araujo, M.E.G.; Esposito, A.; Lu, J.; Hsu, V.; Lucast, L.; De Camilli, P.; Hurley, J.H.; Ballabio, A. Structure of the lysosomal mTORC1-TFEB-Rag-Ragulator megacomplex. *Nature* **2023**, *613*, 196–203. [CrossRef]
130. Sun, N.; Yun, J.; Liu, J.; Malide, D.; Liu, C.; Rovira, I.I.; Holmström, K.M.; Fergusson, M.M.; Yoo, Y.H.; Combs, C.A.; et al. Measuring in vivo mitophagy. *Mol. Cell* **2015**, *60*, 685–696. [CrossRef]
131. Hubens, W.H.G.; Vallbona-Garcia, A.; de Coo, I.F.M.; van Tienen, F.H.J.; Webers, C.A.B.; Smeets, H.J.M.; Gorgels, T.G.M.F. Blood biomarkers for assessment of mitochondrial dysfunction: An expert review. *Mitochondrion* **2022**, *62*, 187–204. [CrossRef] [PubMed]
132. Hetz, C.; Papa, F.R.; Oakes, S.A. The unfolded protein response and cell fate control. *Mol. Cell* **2018**, *69*, 169–181. [CrossRef] [PubMed]
133. Sahin, E.; Bagci, R.; Aykanat, N.E.B.; Kacar, S.; Sahinturk, V. Silymarin attenuated nonalcoholic fatty liver disease through the regulation of endoplasmic reticulum stress proteins GRP78 and XBP-1 in mice. *J. Food Biochem.* **2020**, *44*, e13194. [CrossRef]
134. Benham, A.M. Endoplasmic Reticulum redox pathways: In sickness and in health. *FEBS J.* **2019**, *286*, 311–321. [CrossRef] [PubMed]
135. Sicari, D.; Delaunay-Moisan, A.; Combettes, L.; Chevet, E.; Igbaria, A. A guide to assessing endoplasmic reticulum homeostasis and stress in mammalian systems. *FEBS J.* **2020**, *287*, 27–42. [CrossRef]
136. Tundo, G.R.; Sbardella, D.; Santoro, A.M.; Coletta, A.; Oddone, F.; Grasso, G.; Milardi, D.; Lacal, P.M.; Marini, S.; Purrello, R.; et al. The proteasome as a druggable target with multiple therapeutic potentialities: Cutting and non-cutting edges. *Pharmacol. Ther.* **2020**, *213*, 107579. [CrossRef]
137. Cyran, M.; Zhitkovich, A. HIF1, HSF1, and NRF2: Oxidant-responsive trio raising cellular defenses and engaging immune system. *Chem. Res. Toxicol.* **2022**, *35*, 1400–1423. [CrossRef]
138. Leestemaker, Y.; Ovaa, H. Tools to investigate the ubiquitin proteasome system. *Drug Discov. Today Technol.* **2017**, *26*, 25–31. [CrossRef]
139. Kammerl, I.E.; Hardy, S.; Flexeder, C.; Urmann, A.; Peierl, J.; Wang, Y.; Vosyka, O.; Frankenberger, M.; Milger, K.; Behr, J.; et al. Activation of immune cell proteasomes in peripheral blood of smokers and COPD patients: Implications for therapy. *Eur. Respir. J.* **2022**, *59*, 2101798. [CrossRef]
140. Milani, N.; Parrott, N.; Ortiz Franyuti, D.; Godoy, P.; Galetin, A.; Gertz, M.; Fowler, S. Application of a gut-liver-on-a-chip device and mechanistic modelling to the quantitative in vitro pharmacokinetic study of mycophenolate mofetil. *Lab A Chip* **2022**, *22*, 2853–2868. [CrossRef]

141. Zoumpoulaki, M.; Chiappetta, G.; Bouvet, J.; John, N.; Schanne, G.; Gehan, P.; Diebolt, S.; Shakir, S.; Quévrain, E.; Mathieu, E.; et al. Kinetic redox shotgun proteomics reveals specific lipopolysaccharide effects on intestinal epithelial cells, mitigated by a Mn superoxide dismutase mimic. *Angew. Chem. Int. Ed.* **2025**, *64*, e202422644. [CrossRef]
142. Reinhard, F.B.M.; Eberhard, D.; Werner, T.; Franken, H.; Childs, D.; Doce, C.; Savitski, M.F.; Huber, W.; Bantscheff, M.; Savitski, M.M.; et al. Thermal proteome profiling monitors ligand interactions with cellular membrane proteins. *Nat. Methods* **2015**, *12*, 1129–1131. [CrossRef]
143. Alexandrov, T. Spatial metabolomics and imaging mass spectrometry in the age of artificial intelligence. *Annu. Rev. Biomed. Data Sci.* **2020**, *3*, 61–87. [CrossRef]
144. Kalopitas, G.; Antza, C.; Doundoulakis, I.; Stabouli, S.; Kotsis, V.; Siargikas, A. The impact of silymarin in individuals with non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Nutrition* **2021**, *83*, 111092. [CrossRef]
145. Li, S.; Duan, F.; Li, S.; Lu, B. Administration of silymarin in NAFLD/NASH: A systematic review and meta-analysis. *Ann. Hepatol.* **2024**, *29*, 101174. [CrossRef]
146. Caussy, C.; Alquiraish, M.H.; Nguyen, P.; Hernandez, C.; Cepin, S.; Fortney, L.E.; Ehman, R.L.; Middleton, M.S.; Sirlin, C.B. Optimal threshold of controlled attenuation parameter with MRI-PDFF as the gold standard for the detection of hepatic steatosis. *Hepatology* **2018**, *67*, 1348–1359. [CrossRef] [PubMed]
147. Wah Kheong, C.; Nik Mustapha, N.R.; Mahadeva, S. A randomized trial of silymarin for the treatment of nonalcoholic steatohepatitis. *Clin. Gastroenterol. Hepatol.* **2017**, *15*, 1940–1949.e8. [CrossRef] [PubMed]
148. Leung, H.; Long, X.X.; Ni, Y.; Cheung, C.K.; Rajasekhar, P.; Lau, H.C.H.; Wong, C.Y.; Yu, J.; Ng, S.C.; Chan, F.K.L. Risk assessment with gut microbiome and metabolite markers in NAFLD development. *Sci. Transl. Med.* **2022**, *14*, eabk0855. [CrossRef] [PubMed]
149. Raya Tonetti, F.; Eguileor, A.; Mrdjen, M.; Pathak, V.; Travers, J.; Nagy, L.E.; Llorente, C. Gut-liver axis: Recent concepts in pathophysiology in alcohol-associated liver disease. *Hepatology* **2024**, *80*, 1342–1371. [CrossRef]
150. Sun, J.; Fu, J.; Li, L.; Chen, C.; Wang, H.; Hou, Y.; Xu, Y.; Pi, J. Nrf2 in alcoholic liver disease. *Toxicol. Appl. Pharmacol.* **2018**, *357*, 62–69. [CrossRef]
151. Wang, S.; Yang, R.; Ji, F.; Li, H.; Dong, J.; Chen, W. Sensitive and precise monitoring of phosphatidylethanol in human blood as a biomarker for alcohol intake by ultrasound-assisted dispersive liquid-liquid microextraction combined with liquid chromatography tandem mass spectrometry. *Talanta* **2017**, *166*, 315–320. [CrossRef] [PubMed]
152. Saran, C.; Brouwer, K.L.R. Hepatic bile acid transporters and drug-induced hepatotoxicity. *Toxicol. Pathol.* **2023**, *51*, 405–413. [CrossRef] [PubMed]
153. Vajdi, M.; Adeli, S.; Karimi, A.; Asghariazar, V.; Jazani, A.M.; Azgomi, R.N. The impact of silymarin on inflammation and oxidative stress: A systematic review and meta-analysis of randomized controlled trials. *Int. J. Clin. Pract.* **2025**, *2025*, 3985207. [CrossRef]
154. Shriram, R.G.; Moin, A.; Alotaibi, H.F.; Khafagy, E.-S.; Al Saqr, A.; Abu Lila, A.S.; Charyulu, R.N. Phytosomes as a plausible nano-delivery system for enhanced oral bioavailability and improved hepatoprotective activity of silymarin. *Pharmaceuticals* **2022**, *15*, 790. [CrossRef]
155. Stefan, N.; Cusi, K. A global view of the interplay between non-alcoholic fatty liver disease and diabetes. *Lancet Diabetes Endocrinol.* **2022**, *10*, 284–296. [CrossRef] [PubMed]
156. Saller, R.; Brignoli, R.; Melzer, J.; Meier, R. An updated systematic review with meta-analysis for the clinical evidence of silymarin. *Forsch Komplementmed* **2008**, *15*, 9–20. [CrossRef]
157. Rambaldi, A.; Jacobs, B.P.; Iaquinto, G.; Gluud, C. Milk thistle for alcoholic and/or hepatitis B or C virus liver diseases. *Cochrane Database Syst. Rev.* **2005**, *2*, CD003620. [CrossRef]
158. Sayyad, M.; Sutar, A.D.; Shivhare, K.; Shukla, R.; Flora, S.J.S. Silymarin as a phytopharmaceutical agent: Advances in mechanistic insights, formulation strategies, and pre-clinical applications. *Front. Pharmacol.* **2025**, *16*, 1711653. [CrossRef]
159. Pasquariello, M.; Martinelli, T.; Paris, R.; Moschella, A.; Colombo, R.; Di Bello, A.; Frigerio, J.; Kheloufi, A.; Mirzaabolghasemi, M.A.; Puglisi, D. Exploring the chemotypic variability of *Silybum marianum* and Silybum eburneum by biochemical and genetic characterization. *Front. Plant Sci.* **2025**, *16*, 1584104. [CrossRef]
160. Kim, J.; Han, S.H.; Kim, N.K.; Tran, G.H.; Shim, J.; Chin, J.H.; Lee, K.J. Antioxidant activities and silymarin content of *Silybum marianum* using different extraction methods. *J. Appl. Biol. Chem.* **2024**, *67*, 55. [CrossRef]
161. Walsh, J.; Griffin, B.T.; Clarke, G.; Hyland, N.P. Impact of host and environmental factors on β-glucuronidase enzymatic activity: Implications for gastrointestinal serotonin. *Am. J. Physiol.-Gastrointest. Liver Physiol.* **2020**, *318*, G816–G826. [CrossRef]
162. Meech, R.; Hu, D.G.; McKinnon, R.A.; Mubarokah, S.N.; Haines, A.Z.; Nair, P.C.; Rowland, A.; Mackenzie, P.I. The UDP-glycosyltransferase (UGT) superfamily: New members, new functions, and novel paradigms. *Physiol. Rev.* **2019**, *99*, 1153–1222. [CrossRef]
163. Soeters, P.B.; Wolfe, R.R.; Shenkin, A. Hypoalbuminemia: Pathogenesis and clinical significance. *JPEN J. Parenter. Enter. Nutr.* **2019**, *43*, 181–193. [CrossRef] [PubMed]

164. McCoy, M. Multitissue analysis of absorption, distribution, metabolism, and excretion gene expression: Sex and age effects across human organ systems. *Drug Metab. Dispos.* **2025**, *53*, 100162. [CrossRef]
165. Gufford, B.T.; Chen, G.; Vergara, A.G.; Lazarus, P.; Oberlies, N.H.; Paine, M.F. Milk thistle constituents inhibit raloxifene intestinal glucuronidation: A potential clinically relevant natural product-drug interaction. *Drug Metab. Dispos.* **2015**, *43*, 1353–1359. [CrossRef] [PubMed]
166. Dobiasová, S.; Řehořová, K.; Kučerová, D.; Biedermann, D.; Káňová, K.; Petrásková, L.; Koucká, K.; Václavíková, R.; Valentová, K.; Ruml, T.; et al. Multidrug resistance modulation activity of silybin derivatives and their anti-inflammatory potential. *Antioxidants* **2020**, *9*, 455. [CrossRef]
167. Sorkin, B.C.; Kuszak, A.J.; Bloss, G.; Fukagawa, N.K.; Hoffman, F.A.; Jafari, M.; Barrett, B.; Brown, P.N.; Bushman, F.D.; Casper, S.J.; et al. Improving natural product research translation: From source to clinical trial. *FASEB J.* **2020**, *34*, 41–65. [CrossRef] [PubMed]
168. Loguercio, C.; Andreone, P.; Brisc, C.; Brisc, M.C.; Bugianesi, E.; Chiaramonte, M.; Cursaro, C.; Danila, M.; de Sio, I.; Floreani, A.; et al. Silybin combined with phosphatidylcholine and vitamin E in patients with nonalcoholic fatty liver disease: A randomized controlled trial. *Free. Radic. Biol. Med.* **2012**, *52*, 1658–1665. [CrossRef]
169. Ferenci, P.; Dragosics, B.; Dittrich, H.; Frank, H.; Benda, L.; Lochs, H.; Meryn, S.; Base, W.; Schneider, B. Randomized controlled trial of silymarin treatment in patients with cirrhosis of the liver. *J. Hepatol.* **1989**, *9*, 105–113. [CrossRef]
170. Parés, A.; Planas, R.; Torres, M.; Caballería, J.; Viver, J.M.; 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. *J. Hepatol.* **1998**, *28*, 615–621. [CrossRef]
171. Heinrich, M.; Jalil, B.; Abdel-Tawab, M.; Echeverria, J.; Kulić, Ž.; McGaw, L.J.; Pezzuto, J.M.; Potterat, O.; Wang, J.B. Best practice in the chemical characterisation of extracts used in pharmacological and clinical studies-The ConPhyMP guidelines. *Front. Pharmacol.* **2022**, *13*, 953205. [CrossRef]
172. Abad-Gil, L.; Hurtado-Sánchez, M.C.; Sánchez, M.I.; Acedo-Valenzuela, M.I. Determination of the major bioactive component of *Silybum marianum* in nutricosmetics by a HPLC method with amperometric detection and UAE pretreatment. *Phytochem. Anal.* **2025**, *36*, 934–942. [CrossRef] [PubMed]
173. Chen, Y.-C.; Zane, N.R.; Thakker, D.R.; Wang, S.R. Improving confidence in the determination of free fraction for highly bound drugs using bidirectional equilibrium dialysis. *J. Pharm. Sci.* **2019**, *108*, 1296–1302. [CrossRef]
174. Okour, M.; Brundage, R.C. Modeling enterohepatic circulation. *Curr. Pharmacol. Rep.* **2017**, *3*, 301–313. [CrossRef]
175. Yang, R.; Hirai, T.; Hirama, R.; Tsuchiya, T.; Kamei, K.-I. Integrated gut-liver-on-a-chip platform as an in vitro human model of non-alcoholic fatty liver disease. *Commun. Biol.* **2023**, *6*, 815. [CrossRef]
176. Rex, D.A.B.; Subbannayya, Y.; Modi, P.K.; Palollathil, A.; Gopalakrishnan, L.; Bhandary, Y.P.; Prasad, T.S.K.; Pinto, S.M. Temporal quantitative phosphoproteomics profiling of interleukin-33 signaling network reveals unique modulators of monocyte activation. *Cells* **2022**, *11*, 138. [CrossRef]
177. Mateus, A.; Määttä, T.A.; Savitski, M.M. Thermal proteome profiling for interrogating protein interactions. *Mol. Syst. Biol.* **2020**, *16*, e9232. [CrossRef]
178. Yagishita, Y.; Gatbonton-Schwager, T.N.; McCallum, M.L.; Kensler, T.W. Current landscape of NRF2 biomarkers in clinical trials. *Antioxidants* **2020**, *9*, 716. [CrossRef] [PubMed]
179. Son, M.; Frank, T.; Holst-Hansen, T.; Wang, A.G.; Junkin, M.; Kashaf, S.S.; Ala, T.; Tay, S. Spatiotemporal NF-κB dynamics encodes the position, amplitude, and duration of local immune inputs. *Sci. Adv.* **2022**, *8*, eabn6240. [CrossRef]
180. Iglesias-Aguirre, C.E.; Vallejo, F.; Beltrán, D.; Aguilar-Aguilar, E.; Puigcerver, J.; Alajarín, M.; Berná, J.; Selma, M.V.; Espín, J.C. Lunularin producers versus non-producers: Novel human metabotypes associated with the metabolism of resveratrol by the gut microbiota. *J. Agric. Food Chem.* **2022**, *70*, 10521–10531. [CrossRef] [PubMed]