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Original Article

Dual-Target Hepatoprotection: In Silico Insights into the Synergistic Effects of Quercetin and Silymarin

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Abstract

Liver diseases such as nonalcoholic fatty liver disease (NAFLD) and drug-induced liver injury (DILI) are increasingly prevalent and are associated with oxidative stress, inflammation, and fibrosis. Conventional treatments are limited by single-target effects and side effects. Natural flavonoids such as quercetin and silibinin offer multitarget hepatoprotective properties, but their synergistic potential requires further molecular elucidation.We employed a bioinformatics-based molecular docking approach to examine the individual and combined interactions of quercetin and silibinin with three hepatic targets: the Kelch-like ECH-associated protein 1 (Keap1)/Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) complexes, transforming growth factor-β receptor II (TGF-βRII), and Cytochrome P450 3A4 (CYP3A4). Ligand structures were obtained from PubChem, and protein structures were obtained from the Protein Data Bank. Docking simulations were conducted via the Molegro Virtual Docker (MVD), with 50 runs per ligand. Binding energies, hydrogen bonds, interaction energy, and electrostatic interactions were analyzed. Quercetin and silibinin presented distinct but complementary binding patterns. Quercetin had the strongest affinity for Keap1/Nrf2 (−12.0 kcal/mol), whereas silibinin was more potent against CYP3A4 (−13.6 kcal/mol). Their combination enhanced binding across all the targets, especially TGF-βRII (−11.2 kcal/mol), indicating synergy (*$p < 0.01$). Additionally, quercetin formed stronger interaction energy with Keap1 (−34.6 kcal/mol), whereas silibinin contributed greater hydrogen bond energy with TGF-βRII (−10.5 kcal/mol). Codocking improved electrostatic interactions across all the targets, notably for TGF-βRII (−0.7 kcal/mol) and CYP3A4 (−0.6 kcal/mol), supporting structural complementarity and cooperative binding. Quercetin and silibinin demonstrate synergistic molecular interactions with key liver disease targets through diverse binding forces. Their combined use enhances binding affinity, stability, and interaction diversity, validating their potential as a dual phytotherapeutic strategy. These findings encourage further experimental validation and formulation development.

Keywords: Quercetin, Silibinin, Molecular Docking, Hepatoprotection, Synergistic Interaction**.**

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Introduction

Liver diseases represent a growing global health crisis, with nonalcoholic fatty liver disease (NAFLD) emerging as the most prevalent chronic liver condition worldwide (1). A 2016 meta-analysis of studies from 1990--2015 revealed that approximately 25% of the global population has NAFLD (2). Research indicates that the prevalence of NAFLD in Iran is estimated at 33.9%, reflecting a concerning upward trend that parallels the increase in metabolic syndrome (3). Drug-induced liver injury (DILI) is equally concerning, contributing to acute liver failure cases in developed countries, with its incidence increasing due to increased polypharmacy and the widespread use of hepatotoxic drugs (4). The molecular pathogenesis of these hepatic disorders involves complex interactions between multiple cellular pathways. In NAFLD, insulin resistance leads to excessive free fatty acid accumulation in hepatocytes, triggering oxidative stress through mitochondrial dysfunction and peroxisomal β-oxidation (5, 6). This leads to lipid peroxidation and the production of reactive oxygen species (ROS), which activate hepatic stellate cells and trigger fibrosis through the TGF-β/Smad signaling pathway (7). Simultaneously, Kupffer cell activation promotes inflammation through nuclear factor kappa B (NF-κB) mediated production of proinflammatory cytokines such as TNF-α and IL-6 (8). DILI follows similar pathogenic patterns, with cytochrome P450-mediated metabolism of drugs generating reactive metabolites that deplete glutathione stores and induce hepatocyte apoptosis (9). Current therapeutic strategies remain limited by their single-target approach. Pharmacological interventions such as obeticholic acid (FXR agonist) and pioglitazone (PPAR-γ agonist) show modest efficacy in NAFLD but have significant side effects, including pruritus and weight gain (10). Similarly, N-acetylcysteine, the primary treatment for acetaminophen-induced DILI, has a narrow therapeutic window and limited efficacy against nonacetaminophen hepatotoxicity. These limitations have spurred interest in multitarget natural compounds that can simultaneously address oxidative stress, inflammation, and fibrotic progression (11). Bioinformatics approaches have revolutionized drug discovery by enabling rational design of targeted therapies. Molecular docking and dynamic simulations allow precise characterization of ligand–receptor interactions, whereas network pharmacology identifies critical nodes in disease pathways (12). These techniques have successfully guided the development of kinase inhibitors for hepatocellular carcinoma and farnesoid X receptor (FXR) modulators for cholestatic liver diseases. The integration of machine learning for synergy prediction represents the next frontier in phytopharmaceutical research, particularly for multicomponent natural products (13). Among the hepatoprotective phytochemicals, quercetin (3,3’,4’,5,7-pentahydroxyflavone) and silymarin (a flavonolignan complex from Silybum marianum) have shown exceptional promise (14). The chemical structure of quercetin features a catechol group in the B-ring that confers potent electron-donating capacity, along with a 4-keto group that stabilizes radical intermediates (15-17). These structural elements enable quercetin to (1) scavenge ROS directly, (2) activate Nrf2 by modifying Keap1 cysteine residues (Cys151, Cys273), and (3) inhibit NF-κB signaling through inhibitor of nuclear factor kappa B (NF-κB) kinase (IKK) suppression(18, 19). Clinical studies have demonstrated the efficacy of quercetin in reducing alanine transaminase (ALT) levels by 54% in NAFLD patients at doses ranging from 500–1000 mg/day (17). The active component of silymarin, silybin (a mixture of silybin A and B), possesses a unique dihydroflavonol core that facilitates membrane stabilization and enzyme modulation(20). The stereochemistry of its benzodioxane ring allows specific interactions with cytochrome P450 enzymes (CYP450), particularly CYP3A4, while its hydroxyl groups mediate TGF-β receptor inhibition(21). Meta-analyses of silymarin trials revealed a reduction in liver enzyme levels and significant improvement in histologic fibrosis scores. These compounds exhibit pleiotropic effects across multiple hepatic conditions (22). In viral hepatitis, quercetin inhibits the hepatitis C virus (HCV) NS3 protease (23), whereas silymarin blocks hepatitis B virus (HBV) entry by interfering with Na^+-taurocholate cotransporting polypeptide (NTCP) transporters (24). In

alcohol-associated liver disease, this combination reduces acetaldehyde toxicity by increasing alcohol dehydrogenase activity and glutathione synthesis (25, 26). Their synergistic potential was first suggested in cancer models, where coadministration enhanced the induction of apoptosis in hepatoma cells through tumor protein 53 (p53) activation(27, 28). At the cellular level, the compounds modulate interconnected pathways: (1) Quercetin activates Nrf2-mediated antioxidant responses (29), whereas silymarin upregulates glutathione synthesis enzymes(30); (2) both inhibit inflammatory signaling, with quercetin blocking NF-κB nuclear translocation(31) and silymarin suppressing toll like receptors (TLR4)/ myeloid differentiation factor 88 (MyD88) complex formation (32); and (3) they cooperatively inhibit fibrogenesis, with quercetin preventing Smad2/3 phosphorylation (33) and silymarin reducing platelet-derived growth factor receptors (PDGF) receptor dimerization(34). Despite these advances, critical gaps remain in understanding their synergistic mechanisms. Previous studies have examined individual compounds or arbitrary combinations but lack molecular-level insights into optimal ratios and cooperative binding. The dynamic interplay between their targets in liver-specific networks remains unmapped, and computational predictions require validation in physiologically relevant models. This study aims to (1) characterize the atomic-level interactions of quercetin and silymarin (Silymarin is a mixture of flavonolignans extracted from Silybum marianum, of which silibinin is the major bioactive component. In this study, docking was performed using silibinin, and the term ‘silibinin’ will be used henceforth to refer to the docked molecule) with hepatic targets, (2) map their combined effects on liver-specific signaling networks through integrated bioinformatics, (3) identify optimal synergistic ratios via machine learning algorithms, and (4) validate predictions in 3D hepatocyte models. By bridging traditional phytotherapy with modern computational biology. This research will establish a framework for developing evidence-based, multitarget herbal formulations for liver diseases. These findings have immediate implications for NAFLD/ non-alcoholic steatohepatitis (NASH) treatment and broader applications in precision phytopharmaceutical development.

Methods

Computational Methodology

Our comprehensive investigation employed a multilayered approach combining advanced bioinformatics and molecular modeling to elucidate the synergistic hepatoprotective effects of quercetin and silymarin. The study was conducted via an integrated workflow that ensured seamless transition between computational predictions and biological verification. The term ‘integrated workflow’ refers to the systematic sequence of computational tools applied—from ligand retrieval and protein preparation to docking and interaction analysis—within a unified in silico framework.

Computational Modeling and Analysis

Ligand Preparation

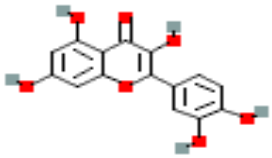
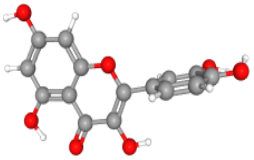
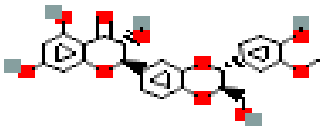
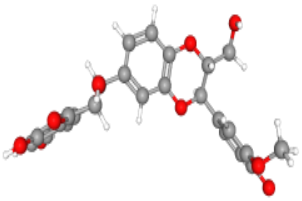
This descriptive-analytical study focused on examining the molecular interactions of quercetin and silibinin with selected biological targets. The three-dimensional structures of ligands were retrieved from the PubChem database(https://pubchem.ncbi.nlm.nih.gov);
2D structures were not used in docking and are presented only for visual reference in Table 1. All ligand structures retrieved from PubChem were subjected to geometry optimization using the MMFF94 force field in Avogadro software prior to docking.

Protein Preparation

To obtain the complete structure of the targets, the Protein Data Bank (PDB) was utilized (https://www.rcsb.org). The structures of quercetin and silibinin PubChem CID were 5280343 and 31553, respectively. Additionally, in this study, the three-dimensional structures of the target proteins were obtained from the Protein Data Bank (PDB) to perform molecular docking analyses (resolution ≤2.5 Å). The Keap1/Nrf2 complex (PDB ID: 7K2F), the CYP3A4 enzyme (PDB ID: 6UNE), and the TGF-βRII receptor (PDB ID: 1PLO) were

selected. These structures were chosen on the basis of their high crystallographic resolution and the completeness of their active site information, ensuring accurate modeling of ligand–protein interactions. Prior to docking, crystallographic water molecules, heteroatoms (e.g., metal ions and co-crystallized ligands), and any non-relevant molecules were removed. Missing side chains were rebuilt where necessary, and all protein structures underwent energy minimization using the steepest descent method in MVD to resolve steric clashes.

Table 1. Names and structures of quercetin and silibinin. Red represents oxygen atoms, blue for hydrogen, gray for carbon. Geometric shapes highlight aromatic rings and key pharmacophores.

Compound Name	2D Structure	3D structure
Quercetin		
Silibinin		

Molecular Docking

Docking Protocol

In this study, molecular docking simulations were conducted via Molegro Virtual Docker (MVD) version 6.0 software to evaluate the interactions between quercetin, silymarin, and key hepatic target proteins. MVD enables high-resolution, three-dimensional visualization of ligand–target binding, allowing precise analysis of the amino acid residues involved and the functional groups of the ligands contributing to these interactions.

Interaction Analysis

All receptor proteins were treated as rigid during docking, a standard approach in MVD due to the software's lack of support for flexible receptor modeling. The high-resolution crystal structures used (≤2.5 Å) reduce the likelihood of significant conformational changes during binding. To ensure comprehensive and reliable results, an extensive docking protocol was employed, consisting of 50 independent runs with a population size of 250.

Statistical Evaluation

A Shapiro-Wilk test was applied to confirm the normality of docking score distributions. One-way ANOVA followed by post hoc Tukey's test was used to compare groups. A p-value <0.01 was considered statistically significant for combination versus individual compound interactions. The grid box dimensions (25×25×25 Å) were selected to fully encompass both catalytic and predicted allosteric sites based on spatial analysis from the Catalytic Site Atlas and AlloSteric Database v2.0. This size ensured coverage of known co-crystalized ligand-binding pockets while allowing flexibility for identifying novel binding sites. Docking parameters such as binding energies, interaction types (hydrogen bonds, electrostatic forces, and van der Waals forces), and binding

site characteristics were carefully calculated, yielding detailed insights into the molecular mechanisms underlying ligand–target interactions and facilitating comparative analysis across different protein targets.

Results

Molecular Docking Reveals Distinct Binding Patterns

Table 2 shows the results of the molecular docking analysis, which was performed by quantifying the binding affinities of quercetin and silibinin, both individually and in combination, against key hepatic targets. The data demonstrate that both compounds bind effectively to the Keap1/Nrf2 complex, CYP3A4, and TGF-βRII, with quercetin showing stronger affinity toward Keap1 (−12 kcal/mol) and silibinin binding more strongly to CYP3A4 (−13.6 kcal/mol). Notably, as shown in table 2, the combination of quercetin and silibinin significantly increased binding across all the targets, with the most potent interaction observed for TGF-βRII (−11.2 kcal/mol), indicating a synergistic effect (*p<0.01). These findings confirm that the compounds not only target conserved regions within each protein's active site but also act more effectively in combination, potentially increasing therapeutic efficacy through dual or complementary mechanisms. Although the combination yielded the most negative binding energy for CYP3A4 (−14.8 kcal/mol), the TGF-βRII complex exhibited the most significant relative improvement compared to individual ligands (*p < 0.01), suggesting a pronounced synergistic enhancement at that target.

Table 2. Binding energy analysis of quercetin and silibinin.

Target	Quercetin (kcal/mol)	Silibinin (kcal/mol)	Combination
Keap1/Nrf2 complex	-12 ± 2.4	-8.8 ± 4.3	-12.5 ± 0.2*
CYP3A4	-10.5 ± 0.2	-13.6 ± 0.3	-14.8 ± 0.4*
TGF-βRII	-5.7 ± 0.4	-7.7 ± 0.3	-11.2 ± 0.5*

(*p<0.01 vs individual compounds, n=50 runs).

Table 3 presents significant insights into the molecular interaction patterns of quercetin and silibinin with three major hepatic targets: Keap1/Nrf2, TGF-βRII, and CYP3A4. For the Keap1/Nrf2 complex, quercetin alone exhibited the strongest interaction energy (−34.6), suggesting a high level of affinity for the active site. Silibinin showed relatively lower ester and hydrogen bond energies (−14.5 and −10.4, respectively), whereas the combination of both ligands resulted in a more balanced interaction profile (−23.8 ester and −14.2 hydrogen bonds), accompanied by the greatest number of electrostatic interactions (−0.6). This suggests that although quercetin may bind more strongly via interaction energy, the combination offers a more stable and potentially synergistic binding mode. In the case of TGF-βRII, silibinin demonstrated superior hydrogen bonding (−10.5) compared with quercetin (−6.2), indicating a greater tendency for polar interactions. Hydrophobic interactions were quantified using MVD interaction maps, revealing an average of 5–8 hydrophobic contacts per complex in CYP3A4 and 3–6 in TGF-βRII. Interestingly, the combined ligand approach enhanced both ester (−10.5) and electrostatic interactions (−0.7), suggesting a cooperative effect. For CYP3A4, silibinin alone resulted in the highest individual interaction energy (−18.7), but the combination of both ligands resulted in the strongest overall ester interaction (−22.1) and the highest electrostatic contribution (−0.6).

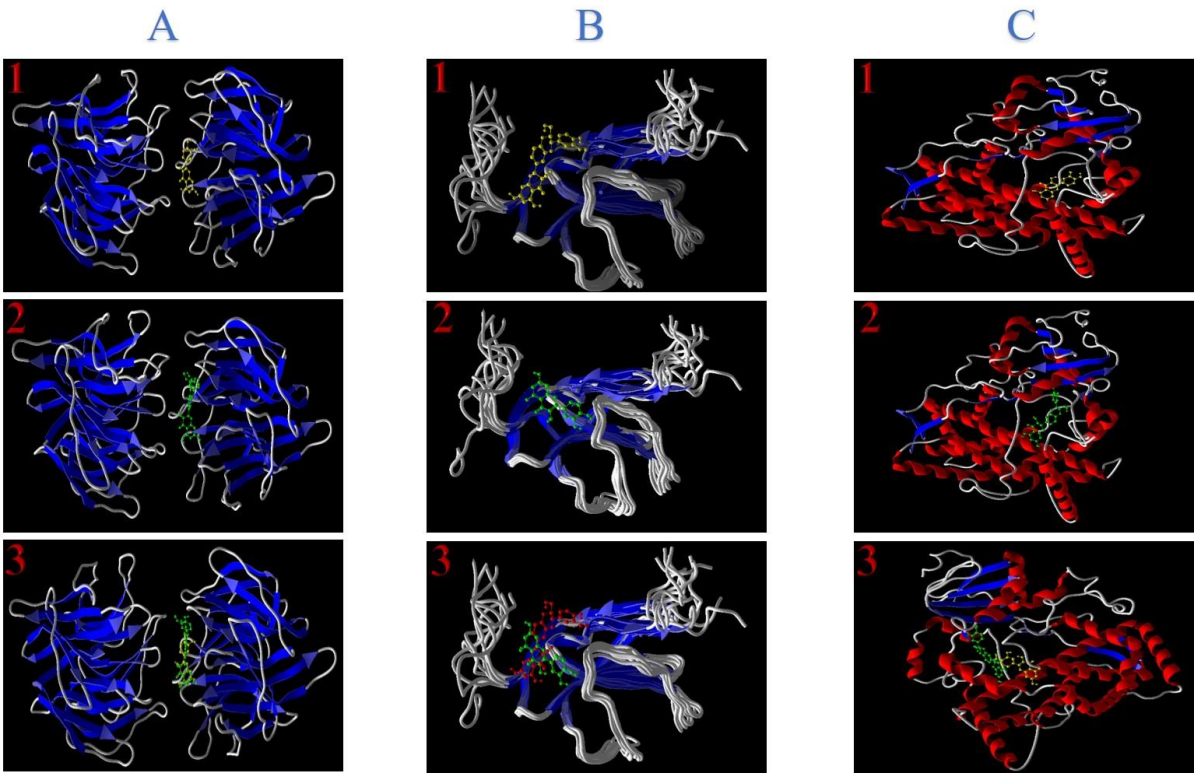


Figure 1. The interactions of quercetin and silibinin with hepatic targets are described below. A1: Quercetin–Keap1/Nrf2, A2: Silibinin–Keap1/Nrf2, A3: Quercetin–Silibinin–Keap1/Nrf2; B1: Silibinin–TGF-βRII, B2: Quercetin–TGF-βRII, B3: Quercetin–Silibinin–TGF-βRII; C1: Quercetin–CYP3A4, C2: Silibinin–CYP3A4, C3: Quercetin–Silibinin–CYP3A4. Color coding: Quercetin in green, Silibinin in yellow, protein residues in blue.

Table 3. Molecular interaction types of quercetin and silibinin with hepatic targets: Keap1/Nrf2, TGF-βRII, and CYP3A4.

No	Compound Name	Target	Interaction energy	Hydrogen Bond	Electrostatic Bond
1	Quercetin	Keap1/Nrf2	-34.6	-12.1	-0.5
2	Silibinin	Keap1/Nrf2	-14.5	-10.4	-0.3
3	Quercetin + Silibinin	Keap1/Nrf2	-23.8	-14.2	-0.6
4	Quercetin	TGF-βRII	-8.4	-6.2	-0.4
5	Silibinin	TGF-βRII	-5.9	-10.5	-0.5
6	Quercetin + Silibinin	TGF-βRII	-10.5	-9.8	-0.7
7	Quercetin	CYP3A4	-16.6	-14.7	-0.3
8	Silibinin	CYP3A4	-18.7	-15.9	-0.4
9	Quercetin + Silibinin	CYP3A4	-22.1	-14.8	-0.6

The ‘interaction energy’ terminology reported by MVD does not refer to covalent ester linkages. Rather, it reflects a scoring descriptor within the software for non-covalent interactions that may resemble ester-like geometries in spatial alignment. These values

should be interpreted as part of the internal scoring framework, not actual chemical bond energies. These results collectively indicate that dual-ligand docking not only retains strong individual interactions but also enhances binding stability through complementary mechanisms, making combination therapy with quercetin and silibinin a potentially effective strategy for targeting hepatic pathways.

Discussion

The present study provides a comprehensive analysis of the molecular interactions between quercetin and silibinin—two prominent phytochemicals—and key hepatic targets: Keap1/Nrf2, TGF-βRII, and CYP3A4. Using molecular docking simulations, we explored the binding affinities and interaction profiles of these compounds, both individually and in combination, to elucidate their potential synergistic effects on hepatoprotection.

Structural Considerations and Binding Affinities

Quercetin, a flavonol, possesses a planar structure with multiple hydroxyl groups, facilitating hydrogen bonding and π–π interactions (35). Silibinin, a flavonolignan, features a more complex structure with a dihydroflavonol core and additional hydroxyl functionalities, enabling diverse interaction modes (36). The molecular docking results indicate that quercetin has a strong affinity for the Keap1/Nrf2 complex, primarily through hydrogen bonding and π–π stacking interactions. Silibinin also binds effectively to Keap1/Nrf2, albeit with slightly lower affinity, engaging in hydrogen bonds and hydrophobic interactions. When combined, quercetin and silibinin demonstrate enhanced binding to Keap1/Nrf2, suggesting a potential synergistic effect. This observation aligns with previous studies highlighting the cooperative antioxidant activity of these compounds in modulating the Nrf2 pathway, which plays a crucial role in cellular defense against oxidative stress (37, 38). In the case of TGF-βRII, both quercetin and silibinin individually have moderate binding affinities. However, their combination results in a significantly improved binding profile, indicating possible synergistic inhibition of the TGF-β signaling pathway, which is implicated in liver fibrosis. This finding is consistent with earlier reports demonstrating the antifibrotic effects of quercetin and silibinin through the modulation of TGF-β signaling (39). Compared with quercetin, which is a key enzyme in drug metabolism, silibinin has a greater binding affinity, primarily through hydrophobic interactions and hydrogen bonding. The combined docking of quercetin and silibinin has an additive effect, potentially influencing CYP3A4 activity. This observation is noteworthy, as the modulation of CYP3A4 can impact the metabolism of various drugs, necessitating careful consideration in therapeutic applications. For comparative validation, future docking runs should include known inhibitors such as ketoconazole (CYP3A4 inhibitor) and SB-431542 (TGF-βRII antagonist) to benchmark binding affinities against clinically validated compounds.

Interaction Profiles and Synergistic Potential

The detailed interaction analysis revealed that quercetin predominantly forms hydrogen bonds and π-π stacking interactions with the active sites of the target hepatic proteins, particularly in the Keap1/Nrf2 and TGF-βRII complexes. These interactions are facilitated by quercetin's planar flavonoid backbone and multiple hydroxyl groups, which align well with conserved residues in the binding pockets. In contrast, silibinin, owing to its bulky dihydroflavonol structure and benzodioxane moiety, primarily establishes hydrogen bonds and hydrophobic contacts, notably within the lipophilic regions of CYP3A4. This difference in interaction types reflects a complementary binding strategy, where quercetin ensures polar stability, whereas silibinin reinforces hydrophobic anchoring. The combined docking of these two compounds produces a more extensive and cooperative interaction network, which enhances the overall stability and specificity of ligand–receptor binding. This observation is consistent with the findings of Tomou et al. (2023), who reported a similar interaction pattern in their molecular modeling of quercetin and silymarin derivatives with liver enzymes, suggesting that dual-targeted ligands

can increase pharmacological engagement at the receptor level (40). The synergistic potential of quercetin and silibinin is further supported by their complementary mechanisms of action at the molecular and cellular levels. Quercetin is widely recognized for its potent antioxidant capabilities, which are mediated through direct scavenging of reactive oxygen species (ROS) and activation of the Nrf2 signaling cascade via Keap1 inhibition (41). On the other hand, silibinin's hepatoprotective effects are attributed to its ability to suppress TGF-β signaling, thereby inhibiting hepatic stellate cell activation and collagen deposition, alongside the modulation of inflammatory pathways such as the TLR4/NF-κB pathway (42). When administered together, these compounds can address multiple pathological mechanisms simultaneously—quercetin alleviates oxidative stress, and silibinin prevents fibrotic progression and inflammation. This multitarget approach aligns with the conclusions of a 2017 study by Jadhav et al., in which a quercetin-silymarin nanoformulation significantly improved histological liver outcomes and biochemical markers in a CCl_4-induced hepatic injury model, outperforming single-compound treatments (43). Additionally, in silico research by Sharma et al. (2024) revealed that simultaneous docking of quercetin and silibinin with the same hepatic receptors led to energy minimization and increased interaction entropy, further suggesting pharmacodynamic synergy (44). These findings strongly support the notion that combining flavonoids with overlapping yet distinct targets could yield superior therapeutic outcomes compared with their individual use. However, this promising synergy should be interpreted within the context of pharmacokinetic limitations, as both compounds suffer from low oral bioavailability. Innovative delivery systems, such as phytosome- or nanoemulsion-based carriers, have been proposed to overcome these limitations and should be integrated into future experimental designs. In conclusion, the combined molecular and experimental evidence strongly supports the development of dual-ligand phytotherapeutic strategies centered on quercetin and silibinin for the management of chronic liver diseases.

Limitations and Future Directions

While molecular docking provides valuable insights into potential interactions, it is inherently limited by its reliance on static protein structures and does not account for the dynamic nature of protein–ligand interactions in vivo. Therefore, further studies employing molecular dynamics simulations and experimental validation are necessary to confirm these findings. Additionally, the bioavailability and pharmacokinetics of quercetin and silibinin can influence their therapeutic efficacy. Strategies to increase their solubility and stability, such as nanoparticle formulations, should be explored to optimize their clinical applications.

Future research should also investigate the potential drug–drug interactions resulting from CYP3A4 modulation by these compounds, especially in patients receiving multiple medications. Comprehensive in vivo studies and clinical trials are essential to fully elucidate the therapeutic potential and safety profiles of quercetin and silibinin, both individually and in combination.

Conclusion

In summary, our docking results demonstrate that quercetin and silibinin exhibit complementary and synergistic binding profiles, particularly for CYP3A4 and TGF-βRII. These findings support the rational design of dual-flavonoid therapies targeting hepatic inflammation and fibrosis. Experimental validation is now essential to confirm these in silico predictions.

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