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RESEARCH

Exploring the drug repurposing potential of silymarin beyond hepatotoxicity treatment through WNT/β-catenin signaling pathway

Silymarin'in WNT/β-katenin yolağı aracılığıyla hepatotoksisite ötesinde terapötik yeniden kullanım potansiyelinin keşfi

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Abstract

Purpose: In this study, the potential of silymarin as a drug for hepatocellular carcinoma (HCC) was evaluated *in situ*.
Materials and Methods: The SwissADME tool was utilized to assess the pharmacokinetic and drug-like properties of silymarin. Molecular docking was performed to model the interaction of silymarin with molecular compounds known to play a role in the WNT/β-catenin pathway and associated with this pathway in HCC. Target proteins (AFP, PIK3CA, β-catenin, PTEN, AAT, AXIN1, GSTM1, GSK3B, PI3K3CA, GSTT1, CCND1, albumin, p53, MET, CTNNB1, and APC) were obtained from the SwissTargetPrediction database. Protein-protein interactions were obtained from the STRING and Cytoscape databases. The PASS platform was used to predict potential bioactivity properties.
Results: The study data revealed that silymarin exhibited the highest binding affinity to the APC protein, with a value of -11.7 Kcal/mol. Although AXIN1 showed the least binding among the studied proteins, with a value of -7.4 Kcal/mol, this can still be considered a good binding affinity.
Conclusion: This study demonstrated the potential of silymarin to inhibit the overactivation of the WNT/β-catenin pathway and identified silymarin as a potential drug candidate for HCC, beyond its hepatoprotective properties. However, further preclinical and clinical studies targeting the WNT/β-catenin pathway are required to confirm the effectiveness and safety of silymarin.
Keywords: Drug repurposing, molecular docking, silymarin, WNT/β-catenin pathway

Öz

Amaç: Bu çalışmada, silymarinin hepatosellüler karsinom (HCC) için bir ilaç olma potansiyeli *in situ* olarak değerlendirilmiştir.
Gereç ve Yöntem: Çalışmada SwissADME, silymarinin farmakokinetik ve ilaç benzeri özelliklerini değerlendirmek için kullanılmıştır. Moleküler docking, silymarinin WNT/β-katenin yolağı ile ilişkili olan ve HCC'de bu yolakla ilişkilendirilen moleküler bileşiklerle etkileşimini modellemek için gerçekleştirilmiştir. Hedef proteinler (AFP, PIK3CA, β-katenin, PTEN, AAT, AXIN1, GSTM1, GSK3B, PI3K3CA, GSTT1, CCND1, albumin, p53, MET, CTNNB1 ve APC) SwissTargetPrediction veritabanından elde edilmiştir. Protein-protein etkileşimleri STRING ve Cytoscape veritabanlarından alınmıştır. PASS platformu, potansiyel biyoaktivite özelliklerini tahmin etmek için kullanılmıştır.
Bulgular: Silymarin en iyi bağlanmayı -11,7 Kcal/mol değeriyle APC proteiniyle göstermiştir. Araştırılan proteinler arasında AXIN1 en az bağlanmayı gösterse de, -7,4 Kcal/mol değeri iyi bir bağlanma afinitesi olarak kabul edilebilir.
Sonuç: Bu çalışmada silymarinin WNT/β-katenin yolağının aşırı aktivasyonunu inhibe etme ve hepatoprotektif özelliklerinin ötesinde HCC için potansiyel bir ilaç adayı olma potansiyeli tanımlanmıştır. Bununla birlikte, silymarinin etkinliğini ve güvenliğini doğrulamak için WNT/β-katenin yolağını hedef alan daha fazla preklinik ve klinik araştırmaya ihtiyaç vardır.
Anahtar kelimeler: İlaç yeniden kullanımı, moleküler docking, silymarin, WNT/β-katenin yolağı

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INTRODUCTION

Drug repurposing, also known as drug repositioning, is an approach aimed at using existing drugs for new therapeutic areas or the treatment of different diseases[1]. The process of drug discovery, from initial research to clinical trials, can take years and result in a significant financial burden This method can significantly alleviate the lengthy and costly process of drug discovery, from initial research to clinical trials, in terms of time and cost. Additionally, the safety profiles and efficacy of existing drugs are often better understood due to extensive research and testing[2]. Therefore, the idea of repurposing existing drugs for new therapeutic areas or different diseases holds great importance.

Silymarin is a phenolic compound found in the seeds of the milk thistle plant (*Silybum marianum*), has been ethnobotanically used for thousands of years to treat liver and gallbladder ailments. It is particularly known for its hepatoprotective efficacy, along with potent antioxidant and anti-inflammatory properties[3,4]. Silymarin's hepatoprotective effect stems from its ability to protect liver cells against toxins and reduce liver inflammation[5]. Furthermore, its antioxidant property mitigates oxidative stress, while it also has the capacity to lower low-density lipoprotein (LDL) cholesterol levels and regulate cholesterol metabolism[6,7]. Recent research has suggested the potential of silymarin to inhibit cancer cell growth and reduce cancer risk[8].

Given its established use in hepatotoxicity, drug repurposing studies are investigating the potential effects of silymarin in the treatment of other liver diseases, as well as in diabetes, cardiovascular diseases, and cancer treatment. Hepatocellular carcinoma (HCC), a significant global health concern, often develops due to liver cirrhosis, with factors such as viral infections, excessive alcohol consumption, obesity, and diabetes also contributing to the risk[9]. The WNT/β-catenin pathway plays a significant role in the molecular pathogenesis of HCC. Overactivation of the WNT/β-catenin pathway and mutations in the TP53 gene are known to contribute to HCC's progression, with processes like angiogenesis, epigenetic changes, and autophagy also playing roles[10,11,12].

Understanding how silymarin affects this pathway can contribute to the development of new strategies for HCC treatment. The potential of silymarin to inhibit the WNT/β-catenin pathway may play a crucial role in HCC treatment by preventing the growth and spread of cancer cells. A detailed examination of this mechanism of action can enhance our understanding of silymarin's anti-cancer efficacy. Additionally, understanding the safety profile and potential side effects of silymarin when used in HCC treatment is vital for clinical applications. This will provide comprehensive information about the efficacy and tolerability of silymarin. More importantly, drug repurposing, or repositioning an existing drug, is often faster and more cost-effective than developing a new drug. The potential of silymarin in HCC treatment can accelerate the drug development process and offer earlier treatment options for patients.

The hypothesis of this study is that silymarin, with its multifaceted pharmacological properties, can inhibit the overactivation of the WNT/β-catenin pathway in HCC, thereby offering a potential therapeutic avenue beyond its known hepatoprotective effects. In this context, the study aims to evaluate the potential effectiveness of silymarin in the treatment of HCC using a drug repurposing approach. Specifically, the interactions of silymarin with HCC cells and its potential inhibitory mechanisms on the WNT/β-catenin pathway have been investigated.

MATERIALS AND METHODS

Druglikeness prediction

Druglikeness is a concept used to predict the pharmacokinetic profile and the potential of a molecule as a drug. This concept is crucial in drug research and development to determine if potential drug candidates will be physiologically active and their effectiveness in the body. Many pharmaceutical companies use druglikeness criteria to assess and optimize new molecules. In this study, evaluating the druglikeness of silymarin is essential for several reasons. First, it helps ascertain its suitability as a therapeutic agent for HCC treatment, particularly regarding bioavailability, metabolism, and potential therapeutic efficacy. Second, understanding silymarin's druglikeness properties aligns with drug repurposing objectives, providing insights into its interaction with the WNT/β-catenin pathway in HCC cells. This evaluation is crucial in establishing the rationale for selecting silymarin for HCC treatment, thereby bridging its pharmacokinetic properties with the study's therapeutic goals.

To investigate silymarin's drug-likeness properties, the SMILES format was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), a widely recognized resource featuring a comprehensive chemical compound databese. This database has provided detailed information on silymarin's chemical structure and interaction potentials with HCC cells. The SMILES format was then uploaded to the SwissADME website (https://www.swissadme.ch/), which offers extensive drug likeness and pharmacokinetic profiling capabilities. SwissADME facilitated the evaluation of silymarin's suitability for HCC treatment by providing information on its absorption, bioavailability, distribution, metabolism, and excretion properties. Four different filters, namely Lipinski's Rule of Five, Ghose filter, Veber filter, and Mudgee filter, were utilized to define the drug-likeness criteria for silymarin. According to Lipinski's Rule of Five, a compound should meet specific criteria, including a molecular weight under 500 daltons, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and a log *P* (octanol-water partition coefficient) not exceeding 5. Compounds violating these criteria three or more times are rejected for not meeting drug-likeness standards. The Ghose filter includes criteria like a molecular weight range of 160 to 480, Log*P* between -0.4 to +5.6, atom count of 20 to 70, and molar refractivity of 40 to 130. The Veber filter evaluates factors such as the number of rotatable bonds (no more than 10) and topological polar surface area (not exceeding 140). The Mudgee filter focuses on criteria like a minimum of 3 rings, at least 18 rigid bonds, and a minimum of 6 rotatable bonds.

Compilation of silymarin-related targets

The SwissTargetPrediction database (https://www.swisstargetprediction.ch/) was used to predict potential targets for silymarin. This predictive tool employs computational methods to identify candidate proteins that might interact with silymarin. To ensure accuracy and standardization, the standard gene names corresponding to these targets were then collected using the UniProt platform. This provided a comprehensive list of proteins related to silymarin.

Molecular docking

Molecular docking serves as a validation method, utilizing computer simulations to predict the binding affinity between receptors and ligands. In this study, docking analyses were conducted which involve silymarin and target proteins. The 3D structures of these target proteins, associated with HCC, were retrieved from the Protein Data Bank (RCSB PDB) database (https://www.rcsb.org). These proteins included alpha-fetoprotein (AFP) (PDB-ID: 7YIM), phosphatidylinositol 3-kinase catalytic subunit alpha (PI3K3CA) (PDB-ID: 7I1C), β-catenin (PDB-ID: 6M90), phosphatase and tensin homolog (PTEN) (PDB-ID: 1D5R), alpha-1-antitrypsin (AAT) (PDB-ID: 1D5S), axis inhibition protein 1 (AXIN1) (PDB-ID: 1DK8), glutathione S-transferase mu 1 (GSTM1) (PDB-ID: 1GT4), glycogen synthase kinase 3 beta (GSK3B) (PDB-ID: 1O9U), glutathione S-transferase theta 1 (GSTT1) (PDB-ID: 2C3N), cyclin D1 (CCND1) (PDB-ID: 2W96), p53 (PDB-ID: 6FFG), hepatocyte growth factor receptor (MET) (PDB-ID: 3ZBX), catenin beta-1 (CTNNB1) (PDB-ID: 4MFU), adenomatous polyposis coli (APC) (PDB-ID: 5G04), phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform (PIK3CA) and albumin (PDB-ID: 6M4R).

To identify the optimal ligand-binding conformations within these target proteins, we used the AutoDock tool. This tool employs a scoring function to evaluate binding conformations based on their free binding energy. Further, the target protein structures were optimized for molecular docking using AutoDock 4.2 software (https://cadd.labshare.cn/cb-dock2/php/blinddock.php). Data related to silymarin were obtained through the online platform DeepDataSource (molinstincts.com).

Building the protein-protein interaction (PPI) network

The interaction relationships among targets were established using the String platform (https://www.string-db.org/). For determining the central nodes and pivotal proteins within the PPI network, Cytoscape was utilized to compute certain centrality metrics without any interference. These metrics include betweenness centrality, closeness centrality, and subgraph centrality. Each of these criteria has been used to determine the structural importance and level of interaction within the network. Specifically, betweenness centrality evaluates the likelihood of a protein acting as a 'bridge' in the interactions between other proteins in the network; closeness centrality assesses a protein's 'accessibility' to other proteins; and subgraph centrality measures how 'central' a protein is within

the local network structure. The combination of these metrics has been employed to identify proteins of key importance in the PPI network, thereby allowing for a more detailed profiling of potential targets with which silymarin interacts.

Proposed bioactivity

It is crucial to determine the potential bioactivity properties of silymarin in order to comprehend its new effects and mechanisms of action. To predict the possible bioactivity properties of silymarin, the PASS platform (http://www.way2drug.com/passonline/index.php) was employed, which can predict 3678 different types of activities with an average accuracy rate of approximately 95% based on the compound's structural formula. The bioactivity predictions made by the PASS platform were used to gain a better understanding of the interactions of silymarin with HCC cells and the WNT/β-catenin pathway. These predictions have formed a critical foundation for determining the potential effects of silymarin on these cells and pathways and examining the relationships of these effects with molecular docking results and other analyses. The bioactivity data obtained have been combined with molecular interaction analyses and PPI network analyses to assess the therapeutic potential of silymarin in HCC treatment and integrate it into the overall findings of the study. This integration has enabled us to understand the effects of silymarin on HCC more comprehensively and to correlate these effects with specific biological pathways and targets.

All data used in this study were obtained from publicly accessible databases such as SwissADME and PubChem. These databases are widely accepted and accessible resources for scientific research. The presented study has adhered to ethical rules and scientific research principles while utilizing data from these databases. Furthermore, throughout all stages of our research, utmost care has been taken to maintain data confidentiality and integrity, and all scientific standards have been considered.

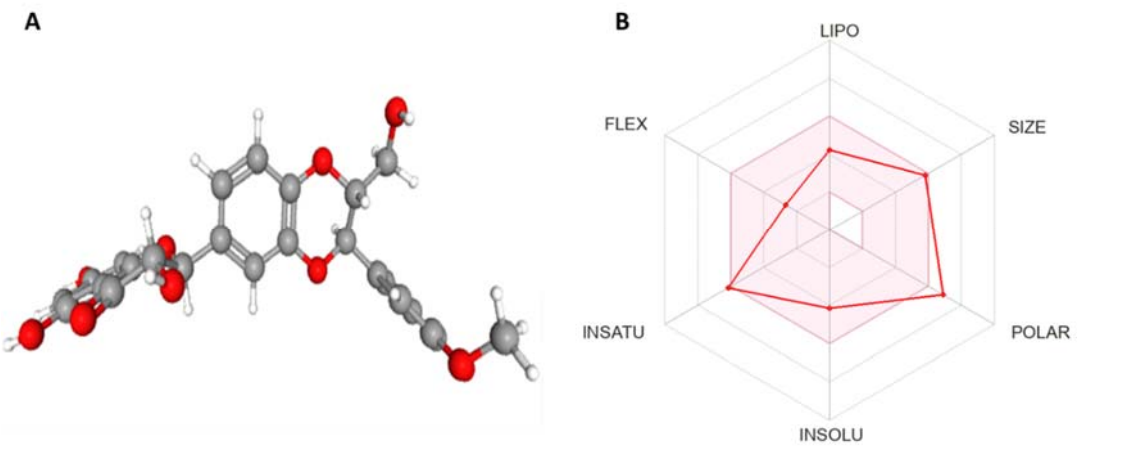

Figure 1. Silymarin 3D conformer (https://pubchem.ncbi.nlm.nih.gov/compound/5213#section=3D-Conformer) SwissADME properties of silymarin (http://www.swissadme.ch/index.php).

RESULTS

SWISS Adme property

The SMILES format of silymarin was obtained by transferring it to the SwissADME website from this source (https://pubchem.ncbi.nlm.nih.gov/compound/ 5213#section=Canonical-SMILES). The three-dimensional configuration of silymarin can be observed in Figure 1A. Silymarin's drug-likeness was comprehensively assessed using filters based on Lipinski, Ghose, Veber, and Mudge. In terms of drug-like properties, it was found to exhibit favorable drug-like properties according to Lipinski's Rule of

Five (lipophilicity value, Log Po/w (iLOGP): 2.79; water solubility values, Log S (ESOL): -4.14; solubility: 3.46e-02 mg/ml; 7.17e-05 mol/l; class: moderately soluble). This analysis could assist in evaluating silymarin as a potential drug candidate (Figure 1B).

Table 1. Interaction of silymarin with target proteins

Receptor	Binding Affinity (Kcal/mol)	Ligand-Molecule Interaction
PTEN	-8.6	Chain A: ALA148 PRO169 SER170 ARG172 ARG173 TYR176 TYR177 SER179 TYR180 LYS183 ASN184 PHE279 ASN323 ASP324
AAT	-8.4	Chain A: LEU124 GLU175 LEU176 ASP177 ASP179 THR180 VAL181 PHE182 ALA183 LEU184 GLU324 ALA325 PRO326 LEU327 LYS328 LEU329 SER330 LYS331 PHE352 LEU353
AXIN1	-7.4	Chain A: PRO166 GLU171 GLU172 ARG174 LEU175 PHE204 CYS208 GLN212 LEU213 ILE214 ASP215 PRO216 MET218
GSTM1	-8.4	Chain A: VAL20 TYR21 Chain B: GLU5 ALA6 GLU12 LEU13 SER14 GLY15 PRO16 ARG96 LYS121 ASN123 VAL124 GLU125 ASP126 GLU127 LEU129 ASN148 LEU150 GLU151
GSK3B	-8.8	Chain A: HIS173 SER174 LEU207 VAL208 ARG209 GLY210 GLU211 ASP233 TYR234 THR235 SER236 SER237 VAL240 PRO325 THR326 ARG328 LEU329 THR330 PRO331
GSTT1	-9.7	Chain A: THR104 THR105 ARG108 GLU139 VAL142 THR143 LEU146 Chain B: PRO13 ASN49 LEU51 LYS53 VAL54 PRO55 THR65 GLU66 SER67 VAL68 HIS103 ARG107 ALA237 MET238 ARG240
CCND1	-9.5	Chain B: ILE12 GLY15 ALA16 VAL20 LYS35 HIS95 VAL96 ASP97 GLN98 ASP99 ARG101 THR102 LYS142 GLU144 ASN145 LEU147 ASP158 VAL175 VAL176 THR177 TRP179
MET	-9.6	Chain A: ILE1084 GLY1085 ARG1086 GLY1087 VAL1092 LEU1157 TYR1159 MET1160 LYS1161 HIS1162 GLY1163 ASP1164 ASN1167 PHE1168 ASN1171 HIS1174 ARG1208 ASN1209 MET1211 ALA1221 ASP1222 ALA1226 TYR1230
CTNNB1	-8.3	Chain A: MET322 LEU323 SER324 ARG327 ILE362 GLU365 ALA412 SER413 ARG416 PHE491 TYR498 TYR536 ASN539 ILE540 GLY541 ASP542 GLY543 ARG544 PHE548 GLU552 ARG555 ILE556
APC	-11.7	Chain F: ALA33 HIS36 Chain H: SER493 HIS494 HIS495 ASN497 THR498 GLU524 ARG527 ILE528 Chain X: SER64 ASN65 ASN67 PRO68 GLU69 LYS76 ASN95 SER98 LYS99 MET102 GLN106 Chain Y: ASP301 PRO302 TYR303
p53	-9.8	Chain A: GLU180 ARG181 GLY187 PRO190 PRO191 GLN192 ASP207 Chain C: THR102 ARG110 LEU111 GLY112 PHE113 HIS115 TYR126 PRO128 ASN131 GLN144 TRP146 ASN268 SER269 PHE270
Albumin	-10.3	Chain A: ASP314 LYS317 ASN318 GLU321 Chain B: PHE509 ILE513 CYS514 ARG521 LYS524 LYS525 MET548 ASP549 PHE551 ALA552 ALA553 VAL555 GLU556 CYS559
β-catenin	-9.1	Chain A: SER201 GLN314 ARG354 PHE355 ASN356 ASP397 PHE398 ASP399 ASP400 GLN437 TYR438 ARG439 ARG477 PHE478 ASP479 ASN480 GLN526 PHE527 ASP528 GLU529
PIK3CA	-9.8	Chain A: ARG6 PHE8 TYR27 ASP30 GLN32 THR233 PRO235 GLY237 ASP238 GLY239 THR240 PHE241 Chain B: TYR26 SER52 ASP53 SER55 PHE56 SER57 TYR63 TYR67
AFP	-9.6	Chain A: LYS242 LEU243 GLN245 LYS246 PHE247 VAL262 HIS266 ILE284 MET285 ILE288 ILE314 ILE315 ASN319 PRO363 GLN364 LEU365 ALA366 VAL367 LYS468 ALA471 CYS472 GLY473 GLY475 ALA476

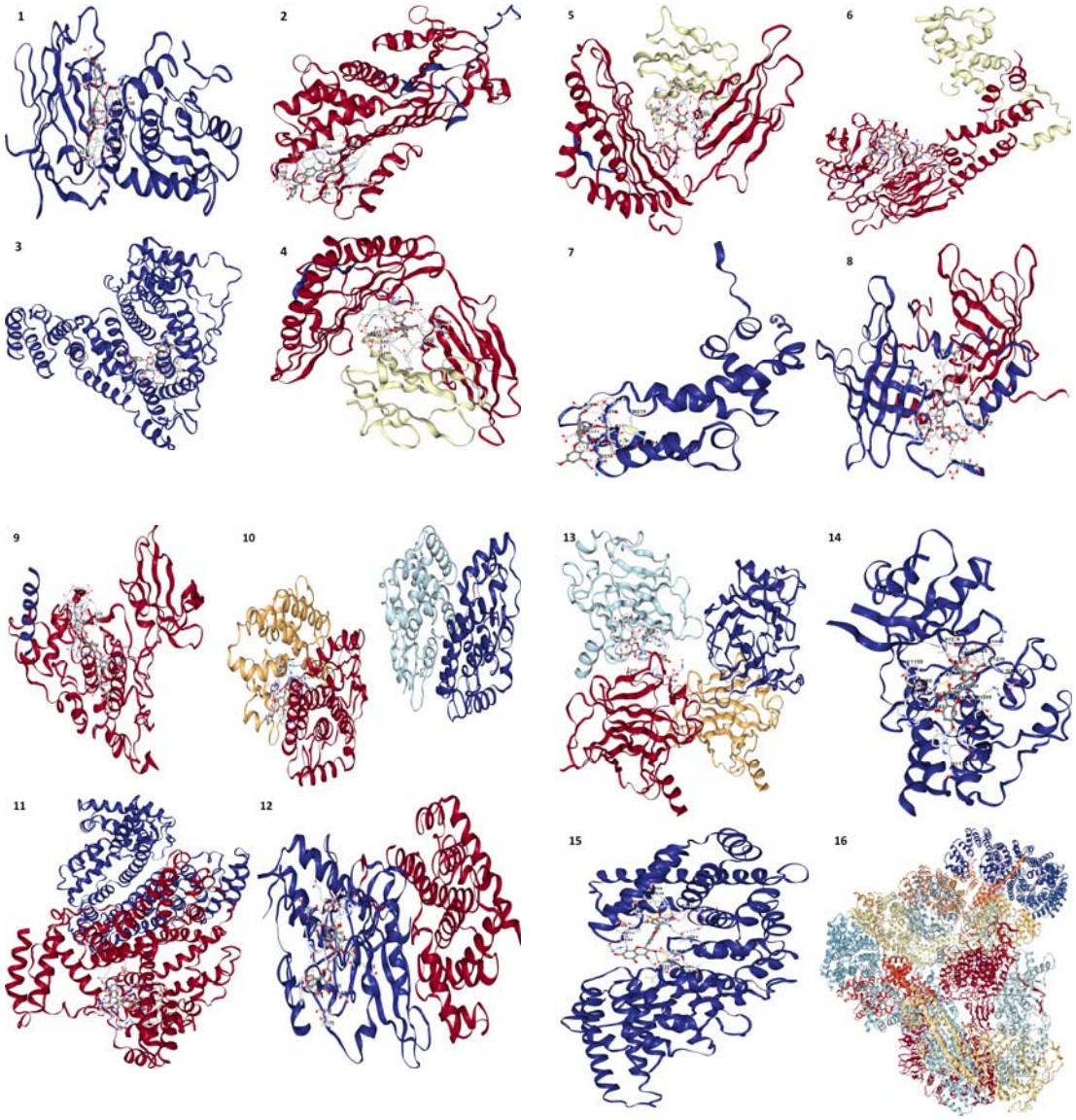

Figure 2. PTEN protein-silymarin interaction (1), AAT protein-silymarin interaction (2), AFP protein-silymarin interaction (3), PI3K3CA protein-silymarin interaction (4), PIK3CA protein-silymarin interaction (5), β-catenin protein-silymarin interaction (6), AXIN1 protein-silymarin interaction (7), GSTM1 protein-silymarin interaction (8), GSK3B protein-silymarin interaction (9), GSTT1 protein-silymarin interaction (10), albümin protein-silymarin interaction (11), CCND1 protein-silymarin interaction (12), p53 protein-silymarin interaction (13), MET protein-silymarin interaction (14), CTNNB1 protein-silymarin interaction (15), APC protein-silymarin interaction (16). The residues and bonds in contact reveal the amino acids and bonding patterns between ligands and target proteins. Hydrogen bond interactions are indicated by teal dashed lines. Yellow dashed lines illustrate electrostatic interactions, whereas grey dashed lines depict hydrophobic interactions.

Molecular docking

Fifteen common targets were selected as potential targets of silymarin in the treatment of HCC. Their binding affinity with silymarin are provided in Table 1. Among the selected targets, it was determined that APC protein had the binding energy, with a value of -11.7 Kcal/mol. Additionally, other targets such as p53 (-9.8 Kcal/mol), PI3K3CA (-9.8 Kcal/mol), GSTT1 (-9.7 Kcal/mol), MET (-9.6 Kcal/mol), AFP (-9.6 Kcal/mol), CCND1 (-9.5 Kcal/mol), PIK3CA (-9.3 Kkal/mol) and β-catenin (-9.1 Kcal/mol) also exhibited significantly high binding energies.

The interactions between silymarin and target proteins were assessed using the AutoDock tool, which employs a scoring function based on binding free energies to evaluate binding conformations. Subsequently, the target protein structures were optimized for molecular docking, and the analysis was conducted using AutoDock 4.2 software. (https://cadd.labshare.cn/cb-dock2/php/blinddock.php). The protein-ligand interactions determined through molecular docking analysis are presented in Figure 2

Creating a Protein-Protein Interaction Network (PPI)

The investigation of protein-protein interactions, aiming to identify 15 common targets, was carried out using the String database (https://www.string-db.org/). These interactions were employed to reveal the functional relationships and interactions among the proteins. Subsequently, the data was transferred to Cytoscape v3.7.2 to create a protein-protein interaction (PPI) network consisting of 45 nodes and 57 edges (Figure 3).

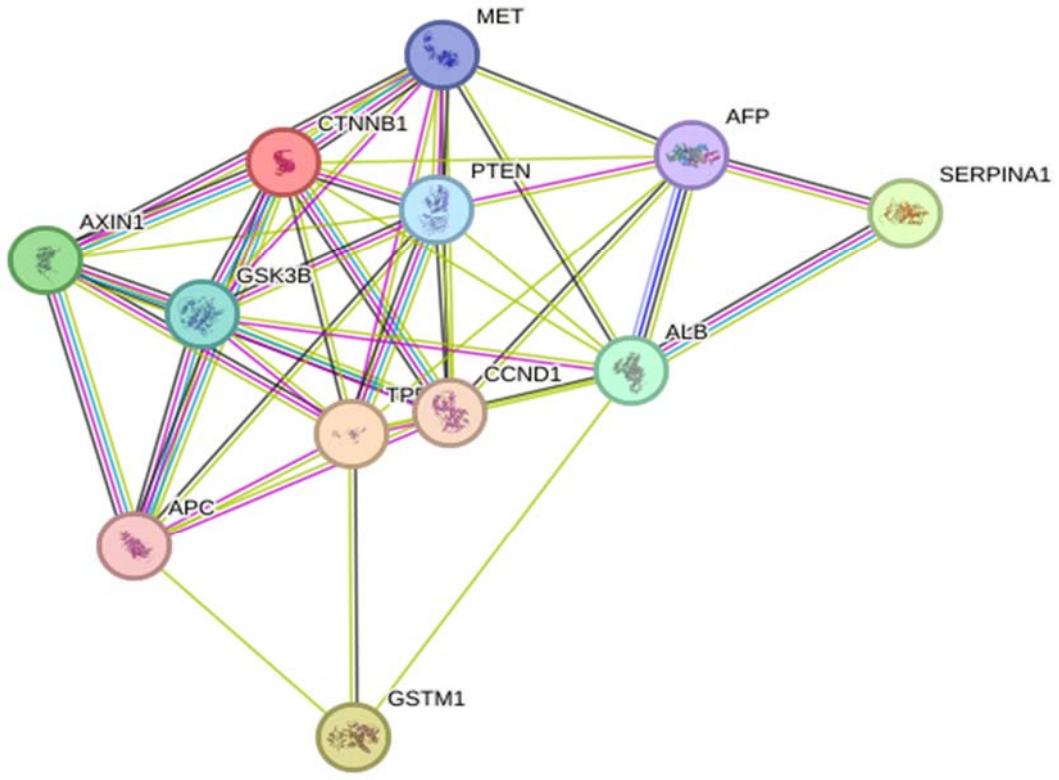

Figure 3. Protein interaction network analysis.

Prediction of bioactivities

Bioactivity prediction was conducted using the platform available at "http://www.way2drug.com/passonline/predict.php." The predictive model for bioactivity spectrum relies on a Bayesian approach. Within this tool, predictions are made for the Pa:Pi (active to inactive ratio) at three distinct threshold levels: Pa > 30%, Pa > 50%, and Pa > 70%. The outcomes of these predictions are expressed in terms of Pa (probability of activity) and Pi (probability of inactivity). Molecules surpassing a Pa value of 0.7 are deemed promising candidates for the specified biological activity (Table 2).

Table 2. The prospective biological activity spectrum associated with silymarin

Pa	Pi	Activities
0.956	0.001	Free radical scavenger
0.957	0.003	Membrane integrity agonist
0.939	0.002	Hepatoprotectant
0.936	0.002	APOA1 expression enhancer
0.914	0.005	TP53 expression enhancer
0.909	0.003	Lipid peroxidase inhibitor
0.859	0.003	Antioxidant
0.857	0.008	HIF1A expression inhibitor
0.844	0.003	HMOX1 expression enhancer
0.809	0.006	CYP1A substrate
0.800	0.005	CYP1A1 substrate
0.798	0.006	Caspase 3 stimulant
0.797	0.004	Chemopreventive
0.783	0.004	Histidine kinase inhibitor
0.785	0.008	CYP3A4 inducer
0.778	0.004	Hepatic disorders treatment
0.765	0.008	Cytostatic
0.750	0.009	CYP3A inducer
0.743	0.005	Antimutagenic
0.704	0.008	Anticarcinogenic

DISCUSSION

In this study, the known hepatoprotective efficacy of silymarin was evaluated *in situ* for hepatocellular carcinoma (HCC) using a drug repurposing approach. Molecular docking studies were employed to understand how a molecule affects a target protein. It is well-known that the overactivation of the WNT/β-catenin pathway is associated with HCC[14]. In this study, molecular docking was performed to assess the binding status of silymarin with the 3D structures of proteins associated with the WNT/β-catenin pathway, including AFP, PIK3CA, β-catenin, PTEN, AAT, AXIN1, GSTM1, PI3K3CA, GSK3B, GSTT1, CCND1, albumin, p53, MET, CTNNB1, and APC. In molecular docking studies, "low-energy binding" typically indicates that the ligand binds tightly and strongly to the protein, suggesting a stable

interaction. The study results indicated that all the tested proteins exhibited low-energy binding with silymarin, with APC protein showing the highest binding energy at -11.7 Kcal/mol, and AXIN1 having the lowest binding energy at -7.4 Kcal/mol. However, AXIN1 also demonstrated favorable binding properties with silymarin. According to SwissADME properties, silymarin exhibited drug-like characteristics in accordance with Lipinski's Rule of five. Furthermore, important bioactivity analysis revealed that silymarin displayed the highest scavenger activity at 0.956 Pa. Notably, it exhibited a significantly high effect in enhancing the expression of the tumor suppressor protein TP53 (Pa 0.914). Molecular docking studies confirmed TP53's high binding affinity with silymarin at -9.8 Kcal/mol. Additionally, silymarin demonstrated chemopreventive, anticarcinogenic, and apoptotic (caspase 3 stimulatory) activities with values of 0.797 Pa, 0.704 Pa, and 0.798 Pa, respectively.

To investigate the potential of silymarin's HCC protective effect through WNT/β-catenin inhibition, 15 potential target proteins were selected. Among these proteins, AXIN1 is a part of the WNT/β-catenin signaling pathway. AXIN1 enhances the degradation of β-catenin by allowing its phosphorylation, preventing its nuclear translocation. Therefore, a potential drug should selectively bind to β-catenin to inhibit its abnormal stabilization and nuclear translocation, which could contribute to the overactivation of this pathway[15]. Molecular docking analysis revealed that silymarin exhibited low binding affinity to β-catenin with a value of -9.1 Kcal/mol. APC protein, which demonstrated the best binding energy with silymarin, is one of the main regulators of the WNT/β-catenin signaling pathway. Loss of function of APC protein in HCC can lead to overactivation of the WNT/β-catenin pathway, which is associated with cellular proliferation and cancer development[16]. GSK3B plays a role in inhibiting the WNT/β-catenin signaling pathway, and inhibition of GSK3B in HCC may support the overactivation of WNT/β-catenin pathways[17]. Silymarin displayed good binding energy with GSK3B at -8.8 Kcal/mol. Furthermore, the p53 gene is known as a cancer tumor suppressor and may play a role in controlling the WNT/β-catenin pathway. A molecular docking study reported that silymarin had a binding energy of -7.2 Kcal/mol with the p53 target protein (PDB 1AlU). Additionally, the PIK3CA gene encodes PI3K, an enzyme regulating cellular signaling pathways, which can interact with the WNT/β-catenin pathway. Furthermore, PTEN losses are frequently observed in HCC development and may contribute to the activation of the WNT/β-catenin pathway.

Apart from proteins associated with the WNT/β-catenin signaling pathway, AFP, a well-known indicator of hepatocarcinoma and useful in early diagnosis and treatment response monitoring, displayed a binding energy of -9.6. Serum albumin is also used as a marker for HCC and its binding energy was found to be -9.7[18]. Additionally, GSTT1 and GSTM1 among the target proteins are involved in toxin metabolism and play a significant role in the liver. The absence of these proteins can increase the risk of liver cancer. Therefore, the potential therapeutic agent should have good binding to these proteins, reducing the risk. Silymarin exhibited binding energies of -9.7 and -8.4 for GSTT1 and GSTM1, respectively[19]. Lastly, excessive MET activation can contribute to HCC progression[20]. Targeting this protein could slow down the progression of HCC, and silymarin demonstrated a binding energy of -9.6 with MET.

The results of this study indicate that silymarin should be considered as a potential drug candidate for HCC treatment. Molecular docking studies revealed that silymarin has high binding affinities to critical proteins associated with HCC. Particularly, its strong binding to APC protein, a key regulator of the WNT/β-catenin pathway, suggests that silymarin may inhibit the overactivation of this pathway, potentially preventing HCC development. Moreover, silymarin's high binding energies with proteins such as p53, PIK3CA, GSK3B, and MET indicate its ability to regulate various cellular processes, controlling tumor growth and progression. These findings emphasize the importance of considering silymarin as a drug candidate with multiple targets for HCC treatment.

One limitation of this study is its reliance solely on data obtained from specific bioinformatics platforms and databases. Additionally, the molecular docking studies that form the basis of this research are theoretical models. It should be noted that these methods may not accurately reflect the real interactions in biological systems. Therefore, experimental research is needed to understand how silymarin interacts with different proteins in cellular or in vivo environments. Furthermore, clinical data are required to confirm the efficacy of silymarin in HCC treatment, and issues such as the potential side

effects, dosage, and interactions of silymarin must be considered in clinical treatments. More research on the pharmacokinetics and side effects of silymarin is also necessary. Finally, the limitations concerning the generalizability of the findings and their applicability to other types of cancer or different biological conditions should be considered. These limitations play a significant role in the interpretation and generalization of the study's results and can guide future research.

The interaction of silymarin with specific proteins associated with HCC, as identified in molecular docking studies, suggests potential therapeutic mechanisms. In clinical settings, this could lead to the development of targeted therapies that inhibit key pathways involved in the progression of HCC. The bioavailability and utilization of silymarin in the body is critical factors for its clinical success. Pharmacokinetic studies are essential to optimize its effectiveness. In the treatment of HCC, issues such as drug resistance and the risk of relapse are significant challenges. Understanding how silymarin interacts with HCC cells could provide insights into overcoming these issues. Although the molecular interactions of silymarin with proteins associated with HCC provide a promising foundation, translating these findings into effective and safe clinical treatments requires overcoming a range of challenges, from clinical trials and safety assessments to personalized treatment strategies and regulatory hurdles.

In conclusion, future research should further investigate the efficacy of silymarin in HCC treatment through more comprehensive experiments supported by clinical studies. Additionally, silymarin's pharmacokinetics and safety should be explored in greater detail, especially regarding its potential role in early HCC diagnosis, treatment response monitoring, and risk reduction factors.

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