

Deciphering the Multi-Target Anti-Diabetic Mechanisms of Chrysin Using Integrated Network Pharmacology, Molecular Docking, and Molecular Dynamics Simulations

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Deciphering the Multi-Target Anti-Diabetic Mechanisms of Chrysin Using Integrated Network Pharmacology, Molecular Docking, and Molecular Dynamics Simulations

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Abstract

Introduction: Diabetes mellitus (DM) is a multifactorial metabolic disorder characterized by impaired insulin signaling, chronic inflammation, and oxidative stress, requiring multi-target therapeutic strategies. Chrysin, a naturally occurring flavonoid, has shown potential anti-diabetic activity, but its molecular mechanisms remain insufficiently defined.

Methodology: Chrysin targets were predicted using SwissTargetPrediction, while diabetes-associated genes were retrieved from public disease databases using a relevance score threshold of ≥ 0.7 . Common targets were identified by overlap analysis, followed by protein–protein interaction network construction, Gene Ontology and KEGG enrichment analysis, molecular docking, molecular dynamics simulations, and MM-PBSA binding free energy estimation.

Results: A total of 31 overlapping targets were identified, including INSR, IGF1R, SIRT1, AKT1, GSK3 β , STAT3, TLR4, and PTGS2. Enrichment analysis showed major involvement in insulin signaling, oxidative stress, inflammation, and pathways such as PI3K/Akt, AMPK, MAPK, and NF- κ B. Molecular docking indicated favorable binding of chrysin to GSK3 β , involving Lys85, Cys199, Ile62, and Leu188. MD simulations confirmed the stability of the chrysin–GSK3 β complex over 100 ns, and MM-PBSA analysis showed a favorable binding free energy of -36.68 ± 0.41 kcal/mol, mainly driven by van der Waals and electrostatic interactions.

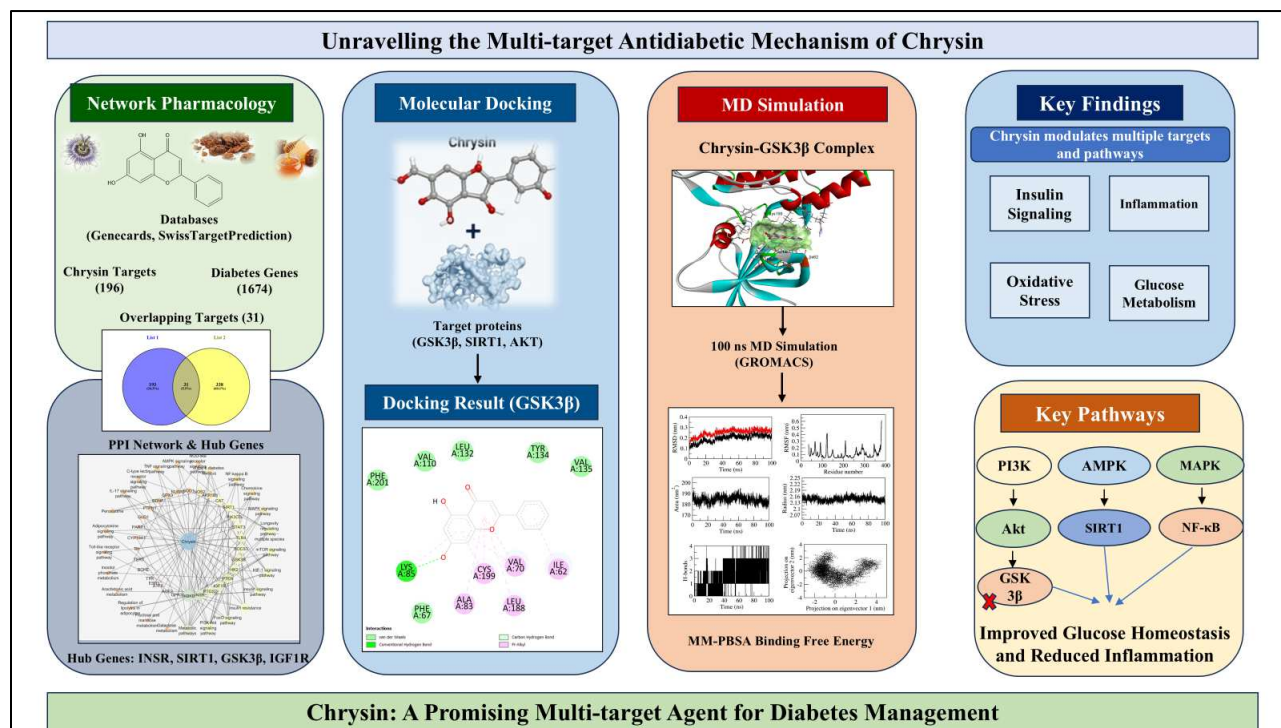
Conclusion: These findings suggest that chrysin may exert anti-diabetic effects through multi-target modulation of key signaling pathways involved in glucose metabolism, inflammation, and oxidative stress. Further experimental validation is needed to confirm these computational predictions.

Keywords: Chrysin, Diabetes Mellitus, Network Pharmacology, Molecular Docking, Molecular Dynamics, Insulin Signaling

Highlights

- Chrysin targets multiple proteins involved in diabetes pathways.
- 31 overlapping targets identified via network pharmacology.
- Key pathways include PI3K/Akt, AMPK, MAPK, and NF- κ B.
- Strong binding of chrysin observed with GSK3 β protein.
- MD simulation confirms stability of chrysin–GSK3 β complex.

Graphical Abstract



1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin resistance, or a combination of both (DeFronzo et al., 2015). The prevalence of diabetes continues to increase worldwide, imposing a substantial burden on healthcare systems and contributing significantly to cardiovascular, renal, neurological, and metabolic complications (Singh et al., 2025). The pathogenesis of type 2 diabetes mellitus (T2DM) is multifactorial and involves complex interactions among impaired insulin signaling, oxidative stress, chronic low-grade inflammation, mitochondrial dysfunction, dysregulated lipid metabolism, and altered cellular energy homeostasis (Galicia-Garcia et al., 2020). Although currently available antidiabetic drugs effectively improve glycemic control, most therapeutic approaches predominantly target a single pathway and are often associated with reduced efficacy during long-term use and undesirable adverse effects. Therefore, the development of multi-target therapeutic strategies capable of modulating interconnected pathological mechanisms has emerged as a promising approach for the management of T2DM (Singh et al., 2025).

Chrysin (5,7-dihydroxyflavone) is a naturally occurring flavonoid widely distributed in honey, propolis, and several medicinal plants (Mani & Natesan, 2018). Numerous experimental studies have demonstrated that chrysin possesses diverse pharmacological activities, including antioxidant, anti-inflammatory, anti-apoptotic, and anti-hyperglycemic effects. Previous *in vitro* and *in vivo* investigations have shown that chrysin improves glucose homeostasis, attenuates insulin resistance, suppresses inflammatory mediators, and mitigates oxidative stress through the modulation of several signaling cascades. Emerging evidence suggests that pathways involving PI3K/Akt, AMPK, MAPK, NF- κ B, and SIRT1 contribute to the biological actions of chrysin. However, owing to the complexity of diabetes pathophysiology, the precise molecular mechanisms and the coordinated interactions between multiple targets and signaling pathways underlying its antidiabetic effects remain incompletely understood (Mapare & Belemkar, 2026).

Conventional drug discovery is primarily based on the “one drug–one target” paradigm, which may not adequately address complex diseases such as T2DM involving multiple dysregulated molecular networks (Chandran et al., 2016). In contrast, network pharmacology integrates systems biology, bioinformatics, and pharmacology to elucidate interactions among bioactive compounds, target proteins, and signaling pathways from a holistic perspective. Furthermore, molecular docking and molecular dynamics simulations provide valuable insights into ligand–protein interactions, structural stability, and binding energetics at the atomic level. The integration of these computational approaches offers a powerful framework for identifying key therapeutic targets and understanding the mechanistic basis of natural compounds exhibiting pleiotropic pharmacological activities (Venkateswaran et al., 2021).

Therefore, the present study aimed to elucidate the molecular mechanisms underlying the anti-diabetic effects of chrysin using an integrated computational strategy combining network pharmacology, protein–protein interaction analysis, functional enrichment analysis, molecular docking, molecular dynamics simulations, and MM-PBSA binding free energy calculations. Furthermore, the study sought to identify key target proteins and signaling pathways involved in the pharmacological actions of chrysin, thereby providing a mechanistic framework for its potential development as a multi-target therapeutic agent for diabetes mellitus (Li et al., 2017a).

2. Materials and Methods

2.1 Identification of Chrysin Targets and Diabetes-Associated Genes

Potential targets of chrysin were retrieved from SwissTargetPrediction (<http://www.swisstargetprediction.ch/>) and PubChem databases based on the canonical SMILES structure of chrysin. SwissTargetPrediction predicts putative protein targets by combining two-dimensional and three-dimensional structural similarities with experimentally validated ligands. Diabetes mellitus-associated genes were collected from GeneCards, DisGeNET, and MalaCards databases using "Diabetes Mellitus" as the search term. To improve target reliability, only genes with relevance scores ≥ 0.7 were selected from GeneCards. Duplicate genes obtained from different databases were removed, and the common targets shared between chrysin and diabetes mellitus were identified using Venn analysis. These overlapping targets were considered potential therapeutic targets involved in the anti-diabetic activity of chrysin (Rappaport et al., 2013; Safran et al., 2022).

2.2 Protein-Protein Interaction Network Analysis

Protein–protein interaction (PPI) analysis of the overlapping targets was performed using the STRING database (version 12.0; <https://string-db.org/>). The organism was restricted to Homo sapiens, and the minimum required interaction score was set to 0.700 (high confidence). Disconnected nodes were excluded from the network to improve robustness. The resulting interaction network was visualized and analyzed using Cytoscape software (version 3.10.0), allowing the identification of highly interconnected proteins associated with diabetes-related biological processes (Szklarczyk et al., 2025).

2.3 Gene ontology and KEGG enrichment pathway

For an in-depth functional enrichment analysis, the open-source, web-based program ShinyGO version 0.76 (available at <http://bioinformatics.sdstate.edu/go/>) has been utilized. This tool generates a variety of useful visualizations, including hierarchical clustering dendrograms, networks highlighting common terms and pathways, protein interaction networks, gene feature plots, and enriched promoter sequences, using

Ensembl annotations. To investigate the potential causal mechanisms of diabetes mellitus genes, ShinyGO was utilized to carry out Gene Ontology (GO) and KEGG pathway assessments on the interactional genes derived from chrysin that exhibited significant hits. The GO analysis categorized these interconnected mechanisms into three major groups: biological processes (BP), cellular components (CC), and molecular functions (MF), each of which provides insights into different aspects of gene functions and their relationships with the biological context. In addition, the KEGG pathway analysis helped further define the specific biological pathways involved in these mechanisms. To guarantee the accuracy and relevance of the results, the most strongly associated GO and KEGG keywords were quantified based on a p-value threshold of <0.05 . This threshold helped in identifying meaningful pathways and terms that are enriched in the largest sub-network of interactional genes. By focusing on these enriched pathways and biological functions, ShinyGO enables a comprehensive understanding of the molecular mechanisms through which metabolites might influence diabetes-related genes, providing valuable insights for further research into potential diabetes mellitus therapies (Yu et al., 2025).

2.4 Compound–Target–Pathway Network Construction

The network construction and visualization were conducted employing the Cytoscape software (version 3.10.0; <http://www.cytoscape.org>) in conjunction with the STRING database (version 10.0; <http://www.string-db.org>) to better understand the molecular mechanisms involved in DM. Three types of networks were constructed based on the research: (1) Protein-Protein Pathway Network: This network focused on the interactions between various proteins involved in diabetes mellitus pathways. (2) Chrysin-Diabetes mellitus Target Network: This network highlighted the interaction between Chrysin and its potential targets related to diabetes mellitus. (3) Diabetes mellitus Protein-Pathways-Chrysin Network: A more comprehensive network that integrates proteins, pathways, and chrysin to reveal their interconnected roles in diabetes mellitus mechanisms. Topological parameters, including node degree and edge count, were calculated using the Network Analyzer plugin. Nodes with higher degree values were considered central regulators within the network (Li et al., 2017a) (Ge et al., 2019).

2.5 Molecular docking

Molecular docking analysis was performed to investigate the binding affinity and interaction pattern of chrysin with glycogen synthase kinase-3 β (GSK3 β), a key regulator of insulin signaling and glucose metabolism implicated in the pathogenesis of type 2 diabetes mellitus. Based on network topology analysis and biological relevance, GSK3 β was selected as the representative target for molecular interaction studies.

The three-dimensional crystal structure of GSK3 β (PDB ID: 1Q5K) was retrieved from the Protein Data Bank (<https://www.rcsb.org/>). Protein preparation was carried out using Discovery Studio Visualizer

(version 2021), wherein co-crystallized ligands, water molecules, and heteroatoms were removed, followed by the addition of polar hydrogen atoms and assignment of Kollman charges. The chemical structure of chrysin was obtained from the PubChem database and energy-minimized using AutoDock Tools (version 1.5.6). Gasteiger charges were assigned, and rotatable bonds were defined to allow conformational flexibility. Molecular docking simulations were performed using AutoDock Vina. A grid box encompassing the active site residues of GSK3 β was generated with dimensions of 20 $\times$ 20 $\times$ 20 Å, and the exhaustiveness parameter was set to 8 to ensure adequate conformational sampling. Multiple binding poses were generated, and the conformation exhibiting the lowest binding free energy was selected as the optimal docking pose. Protein-ligand interactions, including hydrogen bonding and hydrophobic interactions, were visualized and analyzed using Discovery Studio Visualizer and PyMOL software. To validate the docking protocol, the co-crystallized ligand was re-docked into the active site of GSK3 β , and the root mean square deviation (RMSD) between the experimental and predicted conformations was determined. An RMSD value below 2.0 Å was considered indicative of a reliable and reproducible docking procedure (Joseph et al., 2019).

2.5 Molecular dynamics and simulation

To investigate the dynamic behavior and conformational stability of the chrysin–GSK3 β complex under physiological conditions, molecular dynamics (MD) simulations were performed using GROMACS version 2019.4. The initial coordinates of the protein–ligand complex were obtained from the best docking pose. The GROMOS96 54a7 force field was employed for protein parameterization, while ligand topology files were generated using the PRODRG server. The complex was solvated in a cubic simulation box containing SPC/E water molecules, maintaining a minimum distance of 1.0 nm between the protein surface and the box boundaries. Counter ions (Na⁺ and Cl[−]) were added to neutralize the system and achieve electrostatic stability (Lee & Kim, 2007). Energy minimization was carried out using the steepest descent algorithm for 50,000 steps to eliminate unfavorable steric interactions and obtain a stable starting conformation. Subsequently, the system was equilibrated under canonical (NVT) and isothermal–isobaric (NPT) ensembles for 100 ps each at 300 K and 1 atm pressure using the V-rescale thermostat and Parrinello–Rahman barostat, respectively. Long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) method, and periodic boundary conditions were applied throughout the simulation.

Following equilibration, a 100 ns production run was performed with a time step of 2 fs to evaluate the stability and dynamic properties of the protein–ligand complex. Structural fluctuations and conformational stability were assessed by calculating the root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), and intermolecular hydrogen bond occupancy. Principal component analysis (PCA) was carried out to characterize large-scale collective motions, while free energy landscape (FEL) analysis was employed to identify energetically favorable

1 conformational states sampled during the simulation. Furthermore, the binding affinity of chrysin toward
2 GSK3 β was estimated using the Molecular Mechanics Poisson–Boltzmann Surface Area (MM-PBSA)
3 approach. Individual energy contributions, including van der Waals interactions, electrostatic energy, polar
4 solvation energy, and solvent-accessible surface area energy, were calculated to determine the
5 thermodynamic stability of the protein–ligand complex (Alhassan, 2025).

6 **3. Results and Discussion**

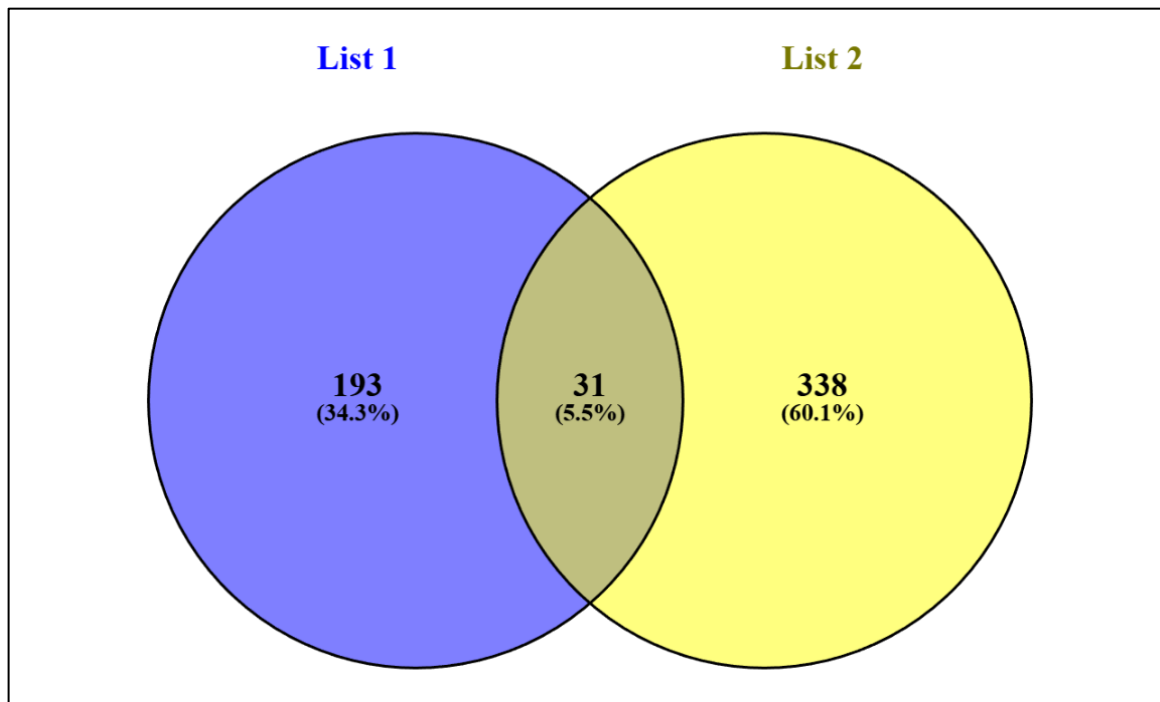
7 ***3.1 Identification of Potential Therapeutic Targets***

8 To elucidate the molecular basis underlying the anti-diabetic activity of chrysin, targets associated with
9 chrysin and diabetes mellitus were retrieved from publicly available databases, followed by Venn analysis
10 to identify common targets. A total of 31 overlapping targets were identified and considered potential
11 therapeutic targets mediating the pharmacological effects of chrysin against diabetes mellitus (Figure 1).

12 The identified targets included enzymes, receptors, transcription factors, and signaling proteins involved in
13 glucose metabolism, insulin signaling, oxidative stress, inflammation, and cellular homeostasis. These
14 targets comprised AKR1B1, CYP19A1, ESR1, PARP1, PTGS2, GSK3B, TTR, IKBKB, BCHE, GLO1,
15 TERT, PIK3CG, INSR, PTPN1, NOS2, IGF1R, GPR35, ACE2, ATF6, BDNF, CAT, GPX3, HK2, NLRP3,
16 PTEN, SIRT1, SOCS3, SOD1, STAT3, TH, and TLR4. The diversity of these targets suggests that chrysin
17 exerts its therapeutic effects through the modulation of multiple interconnected biological processes rather
18 than acting via a single molecular target.

19 Notably, several proteins identified in the present study, including INSR, IGF1R, GSK3B, PTPN1, PTEN,
20 SIRT1, STAT3, IKBKB, NLRP3, and TLR4, have previously been implicated in insulin resistance,
21 inflammatory signaling, oxidative stress, and impaired glucose metabolism, highlighting their potential

1 relevance in the pathogenesis of diabetes mellitus and supporting the multi-target therapeutic profile of
2 chrysin.



3 **Figure 1.** Venn diagram illustrating the overlap between predicted chrysin-associated targets and diabetes
4 mellitus-related genes. A total of 31 common targets were identified and considered potential therapeutic
5 targets involved in the anti-diabetic effects of chrysin.

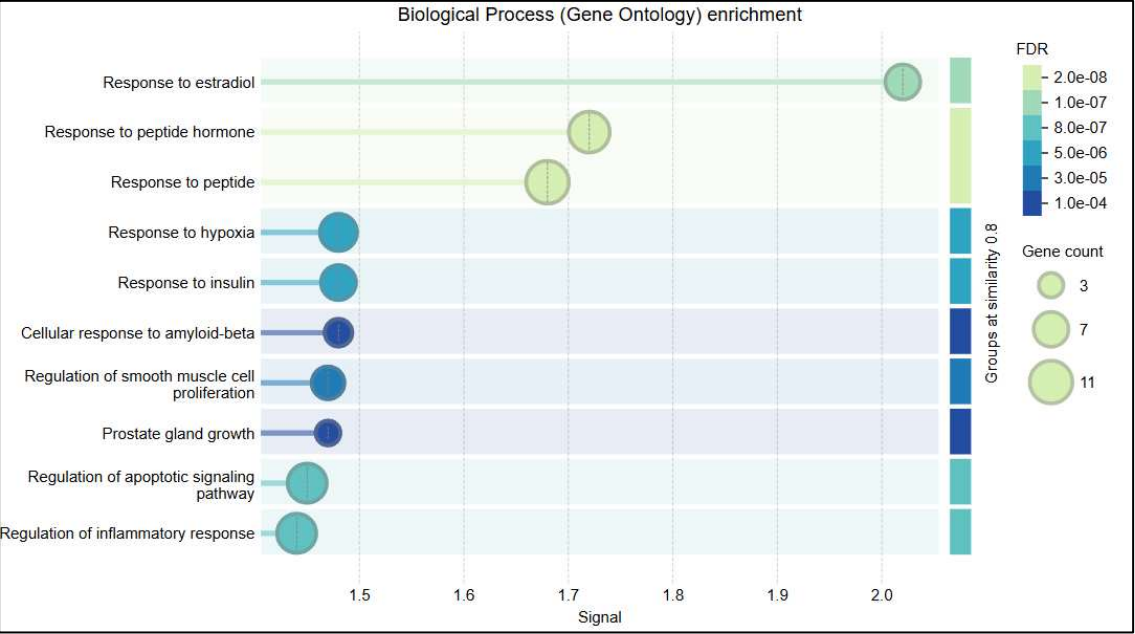
6 **3.2 Gene ontology and Functional enrichment analysis**

7 To gain insight into the biological significance of the 31 overlapping targets, Gene Ontology (GO)
8 enrichment analysis was performed, encompassing biological processes (BP), molecular functions (MF),
9 and cellular components (CC). In addition, disease association analysis was conducted to identify disease-
10 specific functional relationships.

11 **3.2.1 Biological Process Enrichment**

12 GO biological process analysis demonstrated that the identified targets were predominantly enriched in
13 processes related to insulin response, regulation of glucose homeostasis, cellular response to oxidative
14 stress, inflammatory response, and apoptotic signaling (Figure 2A). These processes are critically involved
15 in the development and progression of diabetes mellitus. Impaired insulin signaling and dysregulated
16 glucose metabolism represent key features of insulin resistance, whereas chronic inflammation and
17 oxidative stress contribute to β -cell dysfunction and diabetic complications. Targets such as INSR, IGF1R,
18 GSK3B, PTPN1, SIRT1, and STAT3 are closely associated with insulin sensitivity and glucose utilization,

1 while NOS2, PTGS2, NLRP3, and TLR4 are implicated in inflammatory responses. Furthermore,
2 antioxidant enzymes including CAT, GPX3, and SOD1 participate in cellular defense mechanisms against
3 reactive oxygen species, suggesting that chrysin may exert anti-diabetic effects through the simultaneous
4 modulation of metabolic and oxidative stress pathways.



5 **Figure 2A.** Gene Ontology enrichment analysis (Biological Process) of overlapping targets.

6 **3.2.2 Molecular Function Enrichment**

7 Molecular function analysis revealed significant enrichment in protein kinase activity, insulin receptor
8 binding, antioxidant activity, and enzyme regulatory functions (Figure 2B). Protein kinases such as
9 GSK3 β , PIK3CG, and IKBKB are central regulators of insulin signaling and inflammatory cascades.
10 Dysregulation of these enzymes has been associated with impaired glucose uptake and insulin resistance.

The enrichment of antioxidant-related functions further indicates the involvement of chrysin in mitigating oxidative damage, which is a major contributor to diabetic complications..

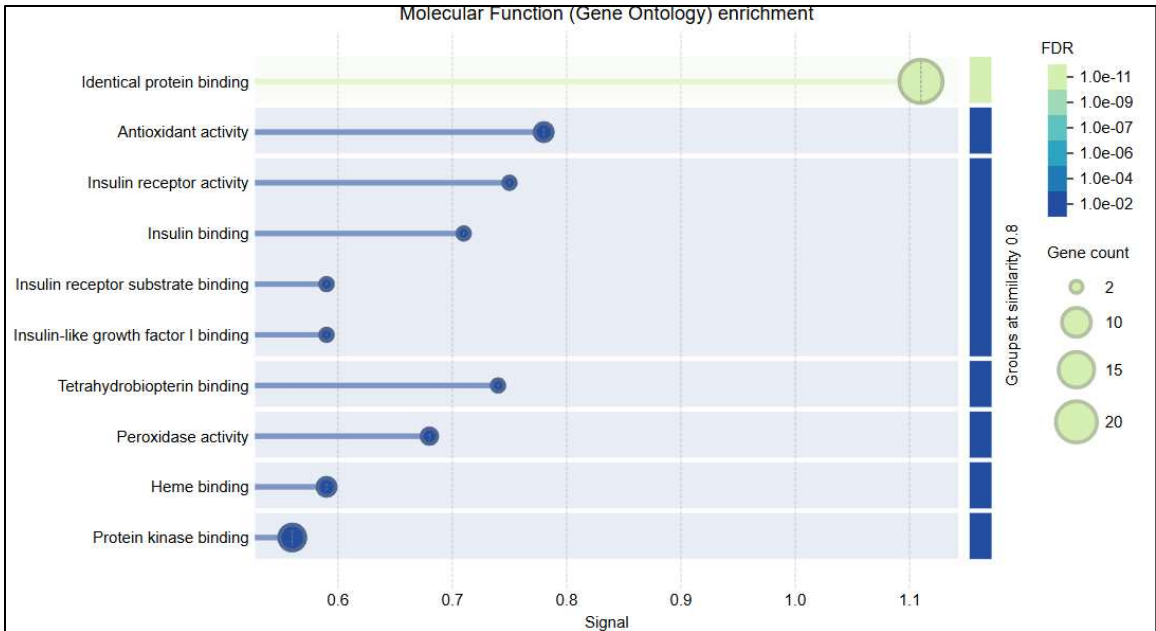


Figure 2B. Molecular Function enrichment analysis of overlapping targets.

3.2.3 Cellular Component Enrichment

Cellular component analysis indicated that the majority of targets were localized within membrane rafts, receptor complexes, cytoplasmic vesicles, and mitochondria (Figure 2C). Membrane-associated proteins

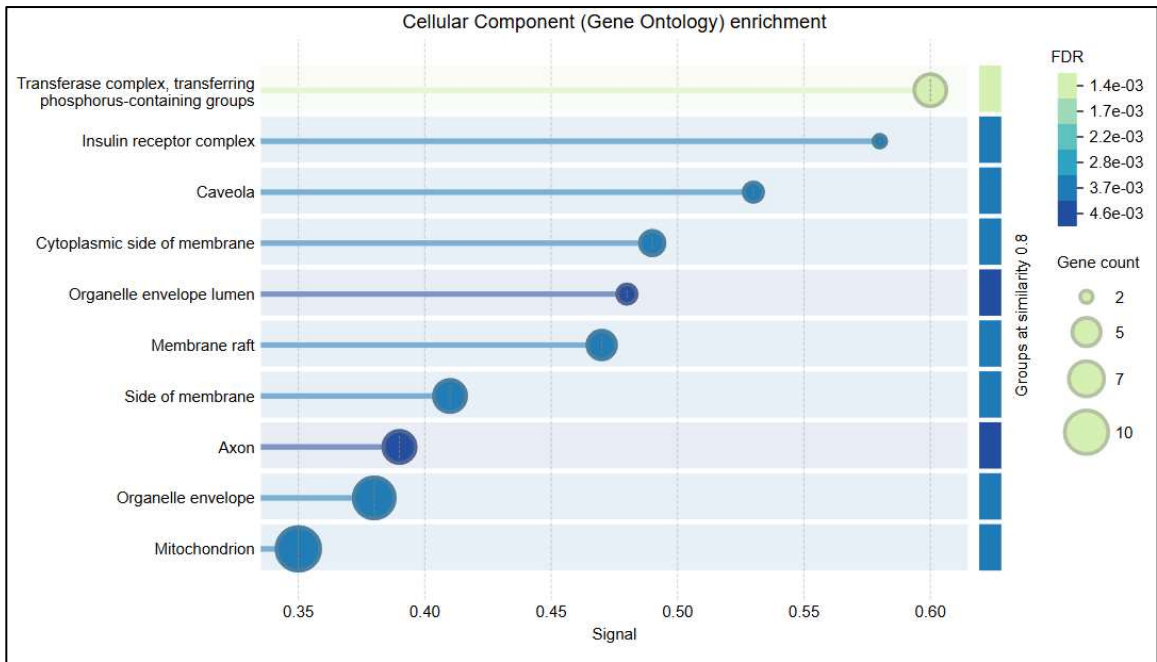


Figure 2C. Cellular Component enrichment analysis of overlapping targets.

such as INSR and IGF1R are essential for initiating insulin-mediated signaling events, whereas mitochondrial localization highlights the importance of energy metabolism and redox homeostasis in diabetes. Mitochondrial dysfunction is a well-recognized contributor to insulin resistance and β -cell impairment, suggesting that chrysin may influence cellular energy balance and oxidative metabolism.

3.2.4 Disease Association Analysis

Disease enrichment analysis revealed strong associations with metabolic disorders, endocrine abnormalities, inflammatory diseases, and oxidative stress-related conditions (Figure 2D). These findings reflect the multifactorial nature of diabetes mellitus and indicate that the identified targets participate in interconnected pathogenic mechanisms involving impaired insulin signaling, chronic inflammation, oxidative stress, and metabolic dysregulation. Collectively, these observations support the multi-target therapeutic potential of chrysin in diabetes mellitus.

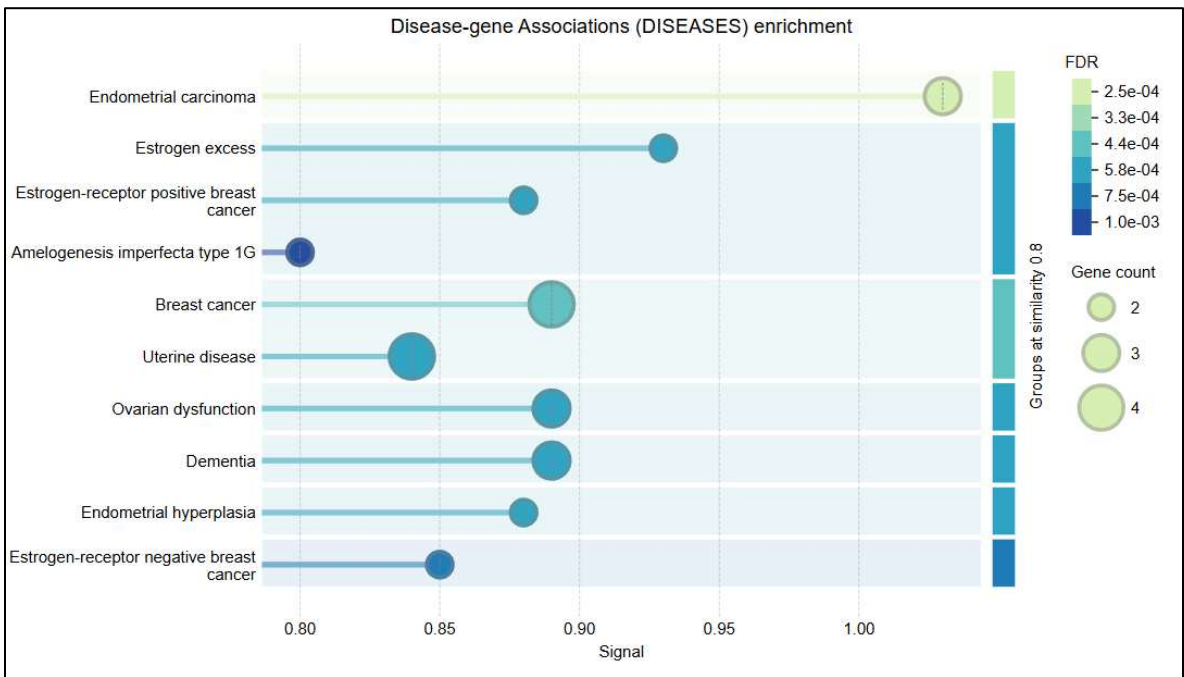


Figure 2D. Disease–gene association enrichment of overlapping targets.

3.2.5 KEGG Pathway Enrichment Analysis

KEGG pathway enrichment analysis demonstrated that the identified targets were significantly enriched in pathways associated with insulin signaling, insulin resistance, inflammation, oxidative stress, and cellular energy metabolism. Among these, the PI3K-Akt signaling pathway emerged as one of the most significantly enriched pathways and plays a crucial role in insulin-mediated glucose uptake and glycogen synthesis. Impairment of this pathway contributes to insulin resistance and hyperglycemia, hallmarks of type 2 diabetes mellitus.

Similarly, the AMPK signaling pathway regulates energy homeostasis and glucose metabolism, while the FoxO signaling pathway is involved in oxidative stress responses and hepatic gluconeogenesis. Enrichment of the NF- κ B and MAPK signaling pathways highlights the contribution of chronic inflammation to diabetes progression. Furthermore, proteins such as INSR, GSK3B, PTPN1, PTEN, STAT3, IKBKB, and SOCS3 participate in insulin signaling and inflammatory cascades, suggesting that chrysin exerts its anti-diabetic effects through coordinated modulation of multiple interconnected pathways rather than a single molecular target. Collectively, these findings support the multi-target therapeutic potential of chrysin in improving insulin sensitivity, reducing inflammation, and restoring glucose homeostasis.

3.3 Network construction analysis

To further elucidate the complex interactions among chrysin, target proteins, and diabetes-associated signaling pathways, a compound–target–pathway network was constructed using Cytoscape 3.10.0 (**Figure 3**). The network consisted of one compound node (chrysin), 31 target nodes, and the top significantly enriched signaling pathway nodes interconnected by multiple edges, illustrating the multi-target and multi-pathway characteristics of chrysin. Topological analysis revealed that several proteins, including INSR, GSK3B, SIRT1, PTPN1, PTEN, STAT3, IKBKB, SOCS3, TLR4, and NLRP3, occupied central positions within the network and exhibited extensive interactions with multiple pathways. These targets are known to regulate diverse biological processes involved in glucose metabolism, insulin sensitivity, inflammatory responses, oxidative stress, and cellular energy homeostasis.

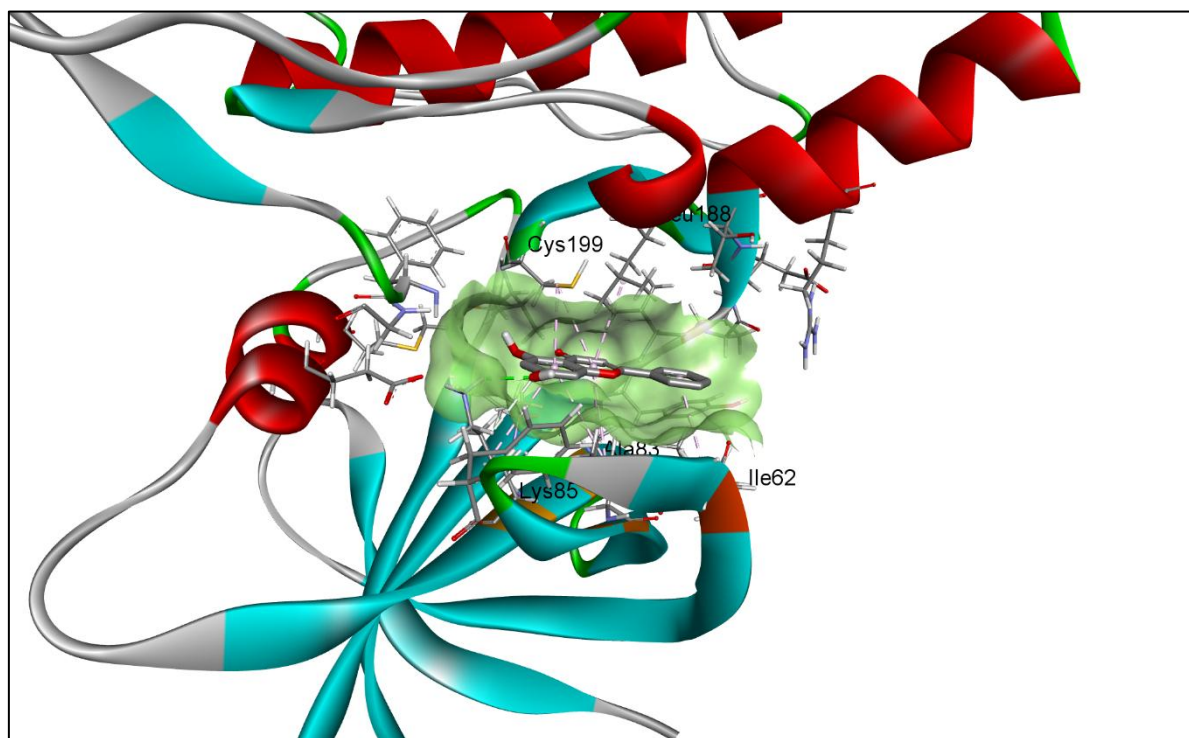
Among the identified targets, INSR serves as the primary mediator of insulin signaling, whereas GSK3 β and PTPN1 negatively regulate glucose utilization and glycogen synthesis, thereby contributing to insulin resistance. Similarly, PTEN modulates PI3K/Akt signaling, while IKBKB, TLR4, STAT3, and NLRP3 participate in inflammatory pathways associated with chronic low-grade inflammation and β -cell dysfunction. Furthermore, SIRT1 plays an important role in cellular energy metabolism and oxidative stress regulation. The target proteins identified in the network were enriched in several diabetes-related pathways, including PI3K-Akt signaling, AMPK signaling, insulin resistance, FoxO signaling, MAPK signaling, HIF-1 signaling, and NF- κ B signaling pathways. These findings suggest that chrysin exerts its anti-diabetic effects through the coordinated regulation of multiple interconnected molecular targets and signaling pathways rather than acting through a single mechanism. The network architecture highlights the systems-level pharmacological actions of chrysin and supports its potential as a multi-target therapeutic agent for the management of type 2 diabetes mellitus.

3.4 Molecular Docking Analysis

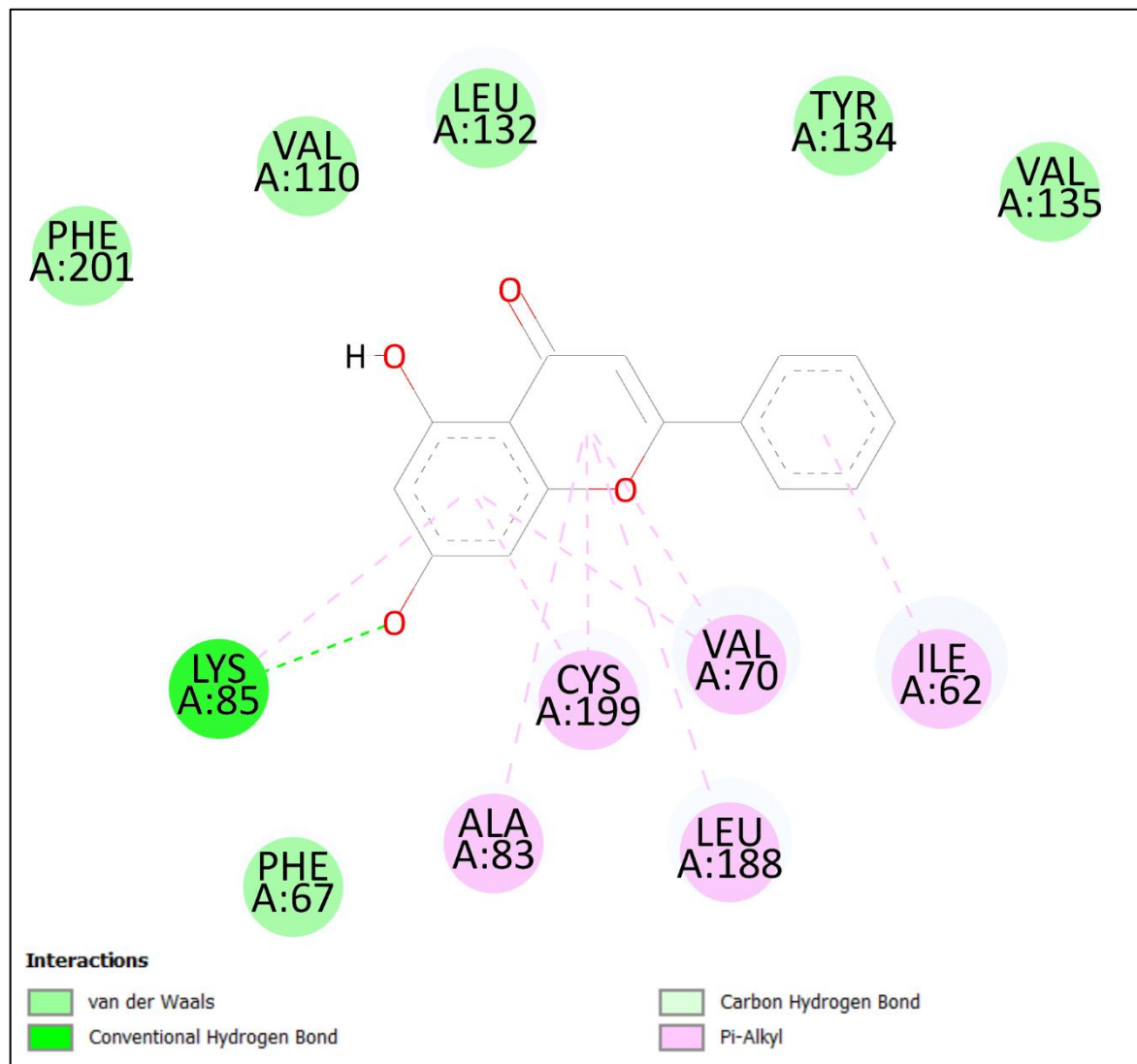
Molecular docking analysis revealed that chrysin exhibited a favorable binding affinity towards the active site of GSK3 β , indicating the formation of a stable protein–ligand complex (**Figure 4**). Detailed interaction analysis demonstrated that chrysin formed hydrogen bonds and hydrophobic interactions with several

1 amino acid residues located within the binding pocket of GSK3 β . Notably, residues such as Lys85, Cys199,
2 Ile62, Leu188, Val70, and Asp200 contributed to the stabilization of the protein–ligand complex through
3 hydrogen bonding and van der Waals interactions. The interaction profile suggested that chrysin occupies
4 the catalytic cavity of GSK3 β and establishes multiple non-covalent interactions that may contribute to the
5 inhibition of kinase activity. Since excessive GSK3 β activity is associated with impaired insulin signaling
6 and reduced glycogen synthesis, the observed binding interactions indicate that chrysin may improve
7 glucose homeostasis through modulation of this key regulatory protein.

8 Collectively, these findings demonstrate the strong binding capability of chrysin toward GSK3 β and provide
9 structural evidence supporting the potential anti-diabetic effects predicted by network pharmacology
10 analysis.



12 **Figure 4.** 3D binding interaction of Chrysin within the active site of the target protein. An analysis revealed
13 key interactions with residues such as Cys199, Lys85, Ile62, and Leu188.



2 **Figure 5.** 2D interaction diagram showing hydrogen bonding and hydrophobic interactions between
3 Chrysin and target protein.

3.5. Molecular dynamics and simulation

The 100 ns molecular dynamics simulation of the Chrysin–GSK3 β complex revealed a highly stable and energetically favorable binding mode supported by consistent structural, dynamic, and thermodynamic parameters. The backbone RMSD (black) stabilized within the first 10–15 ns and remained confined between approximately 0.15 and 0.25 nm for the remainder of the simulation, and the lig+protein RMSD (red) also showed a similar trend in the RMSD within 0.25nm to 0.3nm indicating the absence of significant conformational drift and confirming the structural stability of GSK3 β upon chrysin binding. This observation was further supported by the radius of gyration, which remained narrowly distributed between 2.13 and 2.17 nm, demonstrating sustained compactness of the protein throughout the trajectory. In parallel, the solvent-accessible surface area fluctuated moderately within the range of ~175–195 nm², suggesting that chrysin remains well accommodated within the binding cavity without inducing protein unfolding or excessive solvent exposure. Residue-wise flexibility analysis (RMSF) showed that the majority of GSK3 β residues exhibited low fluctuations, generally below 0.15 nm, particularly within the ligand-binding region. Key residues involved in ligand stabilization—including ILE27, VAL35, ALA48, LYS50, VAL75, LEU97, TYR99, VAL100, THR103, LEU153, CYS164, and ASP165—displayed minimal flexibility, indicating that chrysin binding rigidifies the active-site environment. Higher RMSF values were restricted to loop and terminal regions distant from the binding pocket, reflecting their intrinsic mobility rather than ligand-induced instability.

Hydrogen bond analysis revealed the formation of 1–3 intermolecular hydrogen bonds during most of the simulation, with increased persistence after approximately 30 ns, suggesting progressive stabilization of the ligand orientation. Notably, polar residues such as ASP165 and THR103 intermittently contributed to hydrogen bonding, enhancing binding specificity, although the interaction network was dominated by hydrophobic contacts within the kinase pocket. Essential dynamics analysis using principal component analysis demonstrated that the Chrysin–GSK3 β complex occupies a confined conformational space along the first two eigenvectors (PC1 and PC2), with clear clustering and limited dispersion. This restricted sampling indicates that chrysin binding dampens large-scale collective motions of GSK3 β , which are often required for substrate accommodation and catalytic turnover. Consistent with this, the free energy landscape constructed using RMSD and radius of gyration parameters revealed three dominant low-energy basins, corresponding to thermodynamically stable conformational states, thereby confirming the energetic favorability of the chrysin-bound complex.

MM-PBSA binding free energy calculations further quantified the strength of the interaction, yielding a total binding free energy (ΔG_{bind}) of -36.68 ± 0.41 kcal/mol. The binding was primarily driven by van der Waals (-32.64 ± 0.50 kcal/mol) and electrostatic (-24.42 ± 0.71 kcal/mol) interactions, resulting in a

highly favorable gas-phase interaction energy (-57.06 ± 0.71 kcal/mol). Although polar solvation energy contributed unfavorably (EGB = $+25.15 \pm 0.45$ kcal/mol), this penalty was partially offset by favorable nonpolar solvation (ESURF = -4.77 ± 0.03 kcal/mol), leading to an overall negative solvation contribution (GSOLV = $+20.38 \pm 0.44$ kcal/mol) that was outweighed by strong intermolecular interactions. Residue-wise energy decomposition analysis identified several residues with significant energetic contributions to chrysin binding, including ASP165 (-3.83 ± 0.14 kcal/mol), LEU153 (-2.22 ± 0.05 kcal/mol), TYR99 (-1.87 ± 0.05 kcal/mol), VAL100 (-1.51 ± 0.05 kcal/mol), ILE27 (-1.43 ± 0.07 kcal/mol), LEU97 (-1.29 ± 0.03 kcal/mol), VAL35 (-1.04 ± 0.05 kcal/mol), ALA48 (-1.02 ± 0.02 kcal/mol), and VAL75 (-1.05 ± 0.02 kcal/mol). The ligand itself (UNL347) contributed substantially (-18.77 ± 0.24 kcal/mol), underscoring its central role in complex stabilization. Importantly, the strong contribution of ASP165, a residue critical for GSK3 β catalytic activity, suggests that chrysin directly targets the functional core of the kinase.

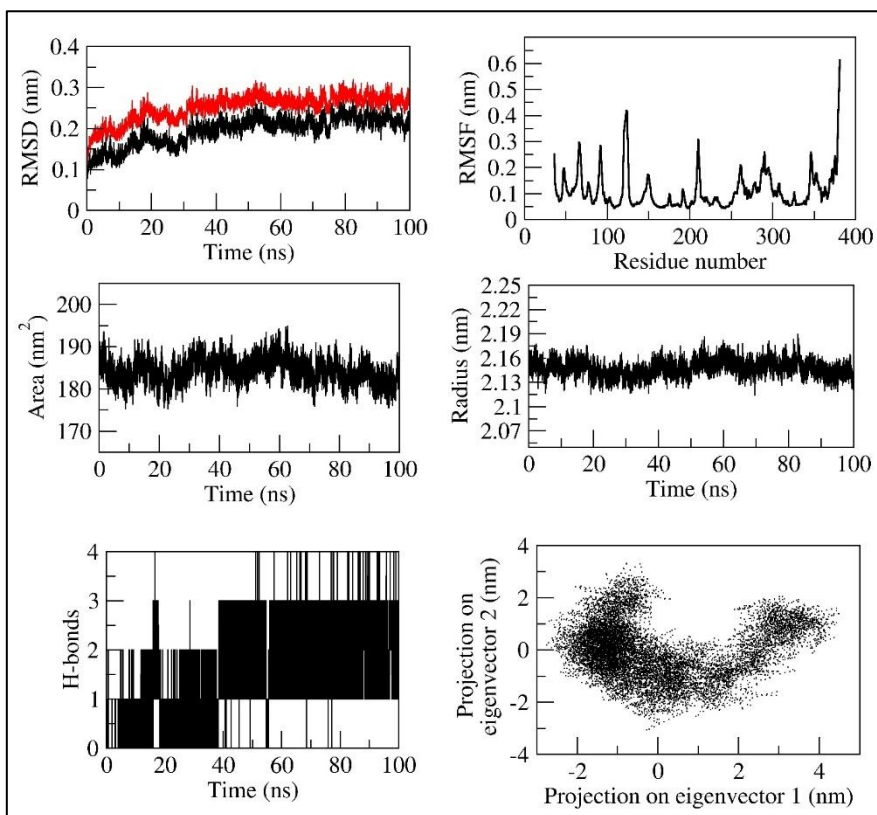


Figure 6. Molecular dynamics simulation analysis of the chrysin–GSK3 β complex over a 100 ns trajectory showing (A) root mean square deviation (RMSD), (B) root mean square fluctuation (RMSF), (C) solvent-accessible surface area (SASA), (D) radius of gyration (Rg), (E) intermolecular hydrogen bond occupancy, and (F) principal component analysis (PCA).

1 These findings confirm that Chrysin forms a stable and energetically favorable complex with the target
2 protein.

3 **4. Discussion**

4 Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder characterized by insulin resistance,
5 impaired glucose utilization, oxidative stress, and chronic low-grade inflammation. Owing to the
6 multifactorial nature of the disease, therapeutic strategies targeting a single protein or signaling pathway
7 often exhibit limited long-term efficacy. Consequently, increasing attention has been directed toward
8 naturally occurring bioactive compounds with pleiotropic pharmacological activities capable of
9 simultaneously modulating multiple pathogenic mechanisms. In the present study, an integrated network
10 pharmacology, molecular docking, molecular dynamics simulation, and MM-PBSA approach was
11 employed to elucidate the molecular basis underlying the anti-diabetic effects of chrysin (Arif et al., 2021;
12 Farkhondeh et al., 2019; Li et al., 2017b; Vybhavi et al., 2025).

13 Network pharmacology analysis identified 31 overlapping targets between chrysin and diabetes mellitus,
14 suggesting that chrysin exerts its effects through a polypharmacological mechanism rather than through a
15 single molecular target. Several hub proteins, including INSR, IGF1R, GSK3 β , SIRT1, STAT3, TLR4,
16 PTGS2, and PTPN1, occupied central positions within the interaction network and are known to regulate
17 insulin signaling, glucose homeostasis, inflammatory responses, and oxidative stress. Dysregulation of
18 these proteins has been implicated in the development of insulin resistance and β -cell dysfunction,
19 indicating that simultaneous modulation of multiple targets may provide superior therapeutic benefits
20 compared with conventional single-target approaches (Lee & Kim, 2007; Li et al., 2017a; Salama et al.,
21 2021).

22 Functional enrichment analysis further demonstrated that the identified targets are involved in biological
23 processes associated with insulin response, inflammatory signaling, oxidative stress, apoptosis, and glucose
24 metabolism. In addition, KEGG pathway analysis revealed significant enrichment of PI3K-Akt, AMPK,
25 MAPK, NF- κ B, FoxO, and insulin resistance pathways. The PI3K-Akt pathway plays a pivotal role in
26 insulin-mediated glucose uptake through GLUT4 translocation, whereas AMPK functions as a cellular
27 energy sensor regulating glucose and lipid metabolism. In contrast, activation of NF- κ B and MAPK
28 signaling pathways contributes to chronic inflammation and insulin resistance. Therefore, the enrichment
29 of these pathways suggests that chrysin may improve glucose homeostasis through coordinated regulation
30 of multiple interconnected signaling cascades.

31 Among the identified targets, GSK3 β was selected for molecular docking and molecular dynamics
32 investigations because of its crucial role in glycogen metabolism and insulin signaling. GSK3 β negatively

1 regulates glycogen synthase activity and contributes to impaired insulin action and hyperglycemia.
2 Persistent activation of GSK3 β has been associated with insulin resistance and β -cell dysfunction, making
3 it an attractive therapeutic target for diabetes management. Molecular docking analysis demonstrated that
4 chrysin exhibited favorable interactions within the catalytic pocket of GSK3 β through hydrogen bonding
5 and hydrophobic interactions involving key residues. These interactions suggest that chrysin may interfere
6 with the catalytic activity of GSK3 β and thereby improve insulin sensitivity and glucose metabolism.

7 The dynamic stability of the chrysin–GSK3 β complex was further evaluated by molecular dynamics
8 simulations. Stable RMSD, radius of gyration, and solvent-accessible surface area profiles indicated
9 preservation of protein structural integrity throughout the simulation period. Likewise, RMSF analysis
10 demonstrated limited fluctuations within the active-site residues, suggesting that chrysin stabilized the
11 catalytic pocket without inducing substantial conformational perturbations. Persistent hydrogen bond
12 interactions and restricted conformational motions observed in principal component analysis further
13 supported the structural stability of the complex. These findings indicate that the docked conformation
14 remains dynamically stable under physiological conditions and strengthen the reliability of the molecular
15 docking results. MM-PBSA analysis provided thermodynamic evidence supporting the favorable
16 interaction between chrysin and GSK3 β . The predominance of van der Waals and electrostatic interactions
17 suggests that both hydrophobic contacts and electrostatic complementarity contribute significantly to
18 complex stabilization. Furthermore, residue decomposition analysis identified several amino acid residues
19 as major contributors to binding energy, including residues located within the catalytic region of GSK3 β .
20 The involvement of these residues indicates that chrysin interacts with functionally important regions of
21 the kinase and may modulate its enzymatic activity.

22 Collectively, the present findings suggest that chrysin exerts anti-diabetic effects through simultaneous
23 modulation of multiple targets and signaling pathways associated with insulin resistance, oxidative stress,
24 inflammation, and energy metabolism. Unlike conventional drugs acting on a single target, the multi-target
25 pharmacological profile of chrysin may provide broader therapeutic benefits in complex metabolic
26 disorders such as T2DM. Nevertheless, the present study is based primarily on computational predictions,
27 and experimental validation is required to confirm the biological relevance of the identified targets and
28 pathways. Furthermore, pharmacokinetic characteristics, bioavailability limitations, and in vivo efficacy of
29 chrysin should be investigated in future studies to facilitate its translational development as a potential anti-
30 diabetic agent.

5. Conclusion

In conclusion, the present study employed an integrated computational approach combining network pharmacology, molecular docking, molecular dynamics simulations, and MM-PBSA analysis to elucidate the molecular mechanisms underlying the anti-diabetic effects of chrysin. Network analysis identified 31 common targets and revealed that chrysin exerts its pharmacological effects through the coordinated regulation of multiple proteins and signaling pathways involved in insulin signaling, glucose metabolism, oxidative stress, inflammation, and cellular energy homeostasis. Functional enrichment analysis highlighted the involvement of key pathways, including PI3K-Akt, AMPK, MAPK, NF- κ B, FoxO, and insulin resistance pathways, supporting the multi-target therapeutic profile of chrysin. Among the identified targets, GSK3 β emerged as a key regulatory protein, and molecular docking studies demonstrated favorable interactions between chrysin and the catalytic pocket of GSK3 β . Furthermore, molecular dynamics simulations and MM-PBSA analysis confirmed the structural stability and thermodynamic favorability of the chrysin–GSK3 β complex, providing dynamic evidence supporting its potential inhibitory activity.

Overall, these findings suggest that chrysin possesses promising multi-target anti-diabetic properties and may represent a potential therapeutic candidate for the management of type 2 diabetes mellitus. However, the present findings are based primarily on computational predictions, and further *in vitro*, *in vivo*, and clinical investigations are warranted to validate the identified targets and pathways and to facilitate the translational development of chrysin as a novel anti-diabetic agent.

Author contributions

GM: Conceptualization; Writing – original draft, and **SB:** Conceptualization; Writing- review and editing.

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Conflict of interests

The authors declare that there is no financial conflict of interest.

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