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Investigation of Cardioprotective Activity of Silybin: Network Pharmacology, Molecular Docking, and *In Vivo* Studies

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The abundant health benefits of silybin are known to benefit people with myocardial infarction (MI). However, their mechanisms of action are not precise. To address this problem, network pharmacology was used to identify the various components that can be utilized to treat this condition, and an *in vivo* study was conducted to evaluate the cardioprotective effect in MI rats. Genes associated with silybin and MI targets were extracted, and overlapping genes between silybin-associated genes and MI targets were identified using Venn diagrams. Using Cytoscape, we built, visualized, and analyzed a network of compounds and genes with pathways. Protein-protein interaction network (PPI), gene ontology (GO) function enrichment, and Kyoto Encyclopedia of Genes, and Genomes (KEGG) pathway enrichment analyses of the core targets were performed to predict its mechanism. A molecular docking study assessed the affinity between silybin and the top three genes. ECG pattern, serum CK-MB, LDH, serum and heart tissue antioxidants, SOD and catalase in isoproterenol-induced MI rats were used to test the cardioprotective effect of silybin. Silybin-related genes (114) and MI-related genes (1800) were identified, and 74 genes overlapped, in which the degrees of AKT1, TNF-α and IL-6 were higher than those of other targets are the disease target precisely. The enrichment of the gene set-based indicated that the PI3K-Akt, TNF-α, IL-17, VEGF, and HIF-1 signaling pathways were significantly involved in the mechanisms of silybin against MI. The QRS complex of the ECG of silybin-treated MI rats was restored to normal ECG and significantly increased serum ($p < 0.0001$***) and heart tissue ($p < 0.0001$***) SOD and serum ($p < 0.001$**) and heart tissue ($p < 0.001$**) catalase compared to MI rats. This study embodies the complex network relationship of multi-target and multiple pathways of silybin in the treatment of MI and provides a novel method for further research on the mechanism of silybin. It has been suggested that silybin alleviates the symptoms of MI by improving antioxidant levels through the PI3K-Akt/HIF-1 pathway.

Introduction

Cardiovascular disease is the leading cause of death worldwide. Among these diseases, myocardial infarction (MI) causes irreversible damage to heart tissue and contributes to a high mortality rate among affected patients.[1,2] Over the past few decades, cardiology has made concerted efforts to identify interventions that can protect the heart from the effects of myocardial infarction. These include using beta-blockers, calcium antagonists, inhibition of the angiotensin-converting enzyme, and antioxidants, although preventive therapy may lead to severe side effects or may require high doses. Given these limitations, alternative remedies using bioactive plant-constituents are gaining research interest as they are often considered more effective and safer.[3] Silybum marianum fruit is rich in silymarin, a flavonolignan with hepatoprotective properties. It's favorable effects on the skin make it a valuable ingredient in cosmetic products. The main active component of milk thistle, silymarin, consists of a mixture of flavonoids in the fruits, seeds, and leaves. The medicinal effects of silymarin complexes include silybin, silydianin, and silychristin. Silymarin has been used to treat alcoholic liver disease, viral hepatitis, and toxin-induced liver diseases. It acts as an insulin sensitizer for anti-ageing, anti-inflammatory, and anti-arthritic activities, anti-Parkinson's disease, hypocholesterolemic and hypolipidemic effects, nephroprotective, immunomodulatory effects,

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and treatment of burns. Silymarin has other interesting properties, including neuroprotection, its use in treating and preventing gastrointestinal and cardiopulmonary problems, and skin protection against harmful UV irradiation. These effects are due to milk thistle's antioxidant and free radical-scavenging properties.[4]

Silybin is the main constituent and bioactive compound with various pharmacological properties, antineoplastic, Alzheimer's disease, inflammation and dyslipidemia, treatment of rosacea, anti-obese, neuroprotective and cardioprotective effect on H9c2 cardiac cells.

As network pharmacology integrates systematic medicine and information science, it holds great promise for drug discovery.[5] It is a method for building a network between protein-compound/disease genes, which can reveal the mechanisms behind the synergy of traditional medicines. This advancement has resulted from a one-target, one-drug paradigm to a multiple-target, multicomponent therapeutic paradigm.

However, the precise molecular pathways underlying the pharmacological and biological activities of silybin remain unclear. As a result of recent advances in bioinformatics, network pharmacology can now be used to uncover and predict biological networks containing compound-protein interactions, protein-protein interactions, and biological signalling activities. Additionally, systematic pharmacological analysis can be used in bioinformatics and statistics to understand and integrate these interacting networks. Considering the antioxidant, hypolipidemic, and anti-inflammatory activities of silybin, we evaluated its potential cardioprotective effects in isoproterenol-induced myocardial infarction models.

Results

Network pharmacology analysis

Potential targets of main active compounds and MI

According to Swiss target prediction, silybin had 114 potential targets. Related genes were selected from the disease gene databases (OMIM, GeneCards, DrugBank, and DisGeNet) using myocardial infarction as a keyword. The results were pooled, and duplicates were deleted. Venn diagrams showed 74 intersections related to myocardial infarction and silybin (Figure 1A). The intersection targets and silybin were imported into Cytoscape to draw a compound–target network diagram (Figure 1B).

Construction and analysis of the PPI network

A PPI network of 74 overlapping genes (Table S1) was constructed using the STRING database. The analysis revealed 74 nodes and 954 edges in the network (Figure 2). We ran the CytoHubba application and extracted data using the MCC calculation method.The top 10 nodes ranked using this method were selected. We identified 41 key genes that belonged to the MCODE cluster 1. In addition, a Venn diagram was created to identify similar hub genes between CytoHubba and MCODE analyses.

As shown in Figure 3 and Table S2, tumor necrosis factor (TNF), albumin (ALB), interleukin-6 (IL6), tumor protein p53 (TP53), AKT serine/threonine kinase 1 (AKT1), vascular endothelial growth factor A (VEGFA), mitogen-activated protein kinase 3 (MAPK3), prostaglandin-endoperoxide synthase 2 (PTGS2), matrix metallopeptidase 9 (MMP9), and caspase 3 (CASP3) were identified as common hub genes that interacted with other proteins at the highest frequency.The Interaction between targets was more vital and the degree value was more significant, indicating an essential role in the PPI network.These results revealed that these targets could be potential targets of silybin in the treatment of MI, which deserves further discussion.

The closeness and betweenness of a network are two critical parameters related to the transmission speed of a signal. The average circuit length connecting one node to another determines the closeness value. The network's interraciality is determined by the shortest path shared by all nodes. An entity with a shorter path is more significant.

Gene ontology (GO) and KEGG pathway analyses

GO enrichment analysis was performed on these targets for DAVID 6.8 to demonstrate the biological functions they serve

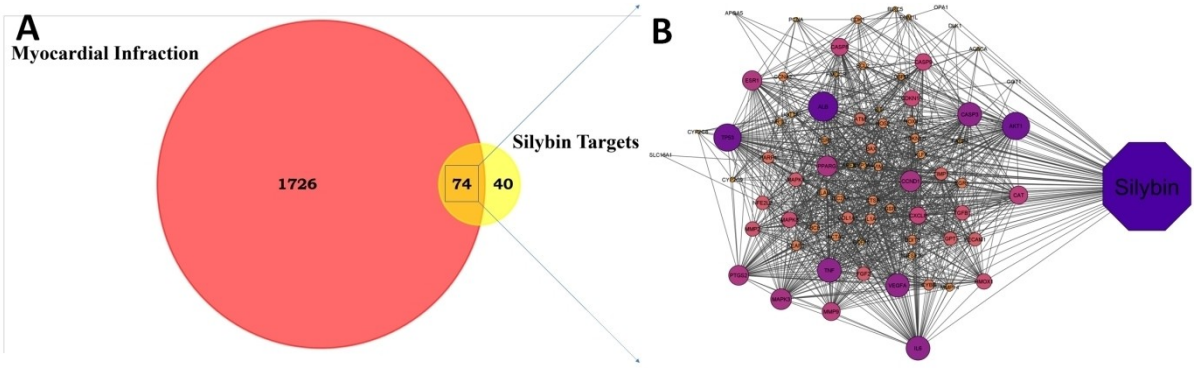

Figure 1. (A) Venn diagram of the silybin and MI targets. (B) Interaction network between silybin and the intersection targets.

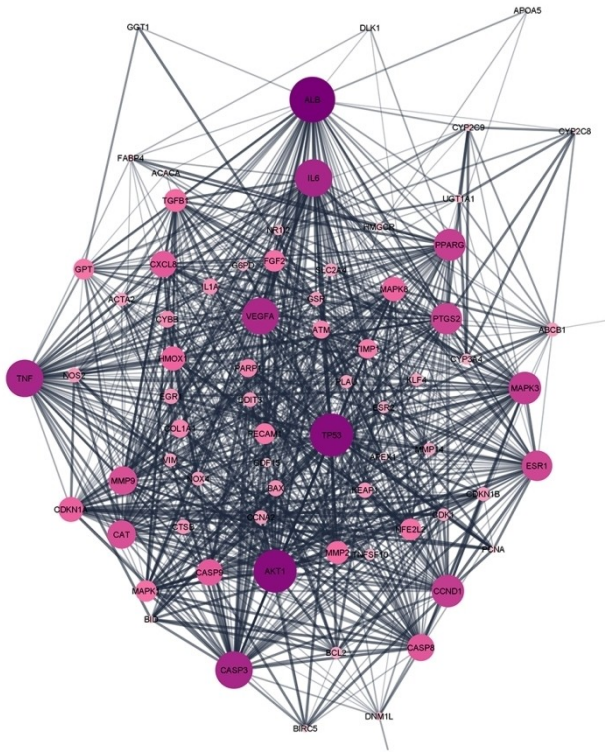

Figure 2. Protein-protein interaction network consists of 74 nodes and 954 edges. Color and size represent the degrees of nodes.

in the treatment of MI with silybin. A total of 114 GO items were obtained through GO analysis ($p < 0.05$), including 98 entries for biological processes, 7 cell component entries, and 9 entries for molecular functions. As shown in Figure 5, the top 10 terms are arranged in ascending order with respect to their p values. The biological processes included positive regulation of the apoptotic process (GO:0043065), negative regulation of the apoptotic process (GO:0043066), positive regulation of peptidyl-serine phosphorylation (GO:0033138), positive regulation of gene expression (GO:0010628), positive regulation of protein phosphorylation (GO:000034), response to glucocorticoids (GO:0051384), negative regulation of cysteine-type endopeptidase activity involved in the apoptotic process (GO:0043154), positive regulation of smooth muscle cell proliferation (GO:0048661), cellular response to hypoxia (GO:0071456), and positive regulation of transcription of the promoter RNA polymerase II (GO:0045944). The cellular components that were predominantly enriched included the cytoplasm (GO:0005737), macromolecular complex (GO:0032991), extracellular region (GO:0005576), and endoplasmic reticulum lumen (GO:0005788). The main molecular functions included identical protein binding (GO:0042802), protease binding (GO:0002020), cytokine activity (GO:0005125), enzyme binding (GO:0019899), and protein phosphatase 2 A binding (GO:0051721). Table S3 and Figure 4 present the top 10 BPs, top 10 CCs, and top 10 MFs according to count.

Signaling pathways and finding of a hub signalling of silybin against MI

KEGG pathway enrichment analysis was then performed (Figure 5). All pathways with a p-value < 0.05 were screened and ranked by p-value. The main pathways enlisted were the proteoglycans in cancer, AGE-RAGE signalling pathway in diabetic complications, IL-17 signaling pathway, TNF signalling pathway, relaxinsignalling pathway, pathways of neurodegeneration, multiple diseases, MAPK signalling pathway, and TGF-beta signalling pathway (Table S4). Furthermore, a cluster analysis using ClueGo indicated that the KEGG-enriched pathways were mainly classified in the IL-17 signaling pathway and TNF signalling pathways (Figure 6).

Component-target-pathway network

To better understand the correlations between silybin, targets, and pathways, a complete network constructed using the top 20 pathways was used to identify 10 hub genes associated with both silybin and other pathways (Figure 7). The topological parameters of the silybin treatment of the MI network were analyzed using the Network Analyzer in Cystoscope 3.6.0 to identify the core components and targets. According to the results of network analysis, IL-6_silybin, AKT1_silybin, and TNF_silybin were predicted to be significant genes based on the degree of connection in the network. The three genes were chosen for molecular docking based on KEGG, PPI, and literature.

Binding affinity

As 74 potential targets were obtained, the top three targets (TNF, AKT1, and IL6) with the highest PPI values were selected as targets, and silybin was used as a ligand for molecular docking[6] to verify the binding efficiency of silybin and its potential targets against MI. The scores for silybin and the positive control (atorvastatin) are compared in Table S5. Following convention, the binding capacity between silybin and proteins was assumed to exist when the binding energy score was greater than −4.25. Scores greater than 5.0 indicate relatively high binding affinity, and scores greater than 7.0 indicate a potent ligand-receptor interaction.[7]

Using docking analysis, silybin was successfully predicted to bind to the three target protein-binding pockets. The interaction binding energy of silybin and atorvastatin with TNF was −6.4 kcal/mol and −5.9 kcal/mol, respectively. The Interaction binding energies of silybin and atorvastatin with IL-6 were −7.6 kcal/mol and −6.4 kcal/mol, respectively. Interaction binding energy of silybin and atorvastatin interaction binding energy with AKT1 −7.7 kcal/mol and −7.3 kcal/mol, respectively. Silybin exhibited binding energies similar to those ofthe standard compound atorvastatin with all three targets. Therefore, this study suggests that silybin binds to the three target proteins and acts as an MI repressor. In addition, to fully understand how silybin acts against MI, it is crucial to understand how these three targets interact. Silybin interacts with

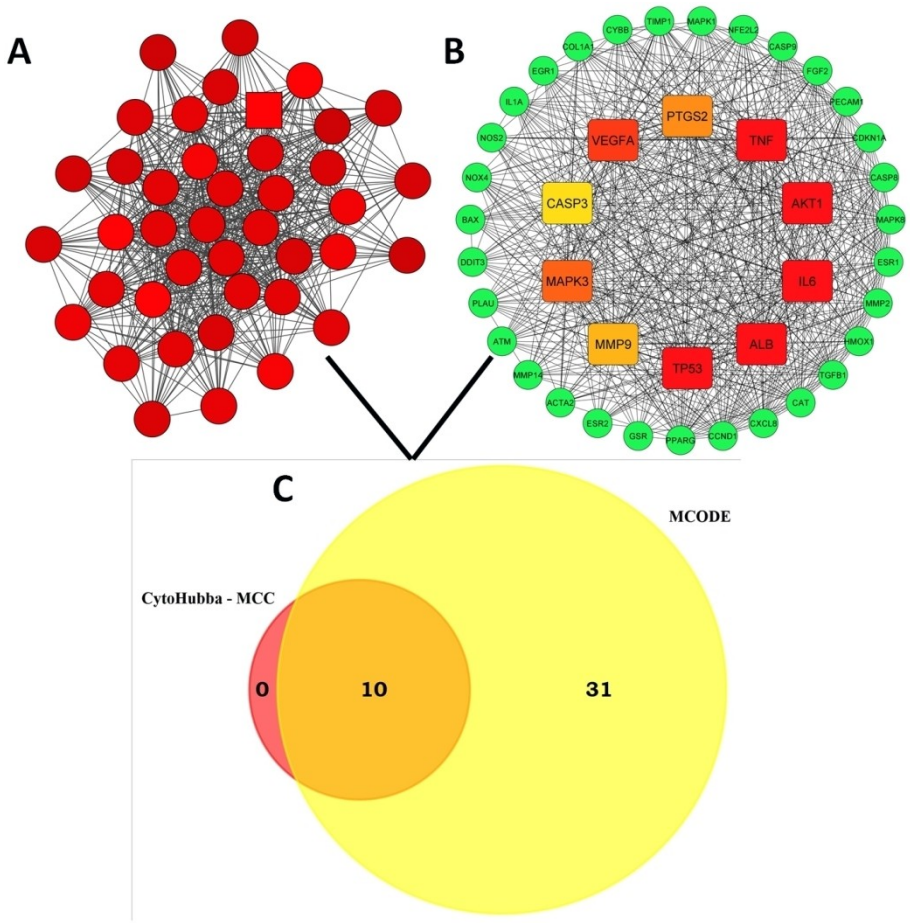

Figure 3. Identification of hub genes of the PPI network using Cytoscape plugins, (A) MCODE and (B) CytoHubba; (C) Identification of common genes from CytoHubba and MCODE.

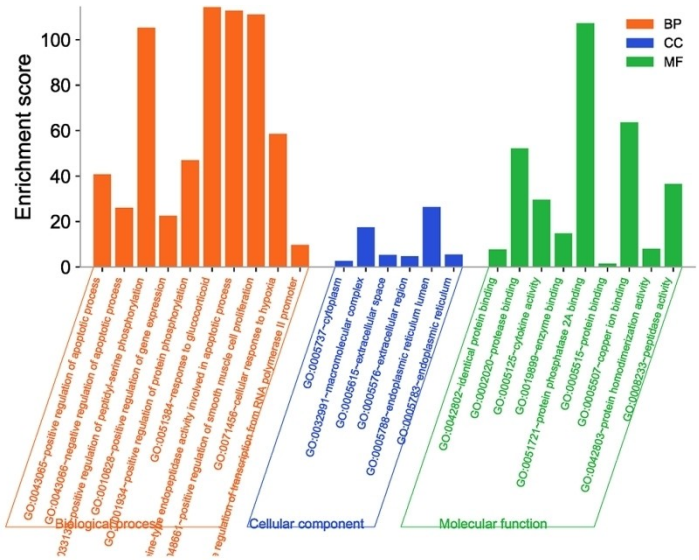

Figure 4. Biological process analysis of active components.

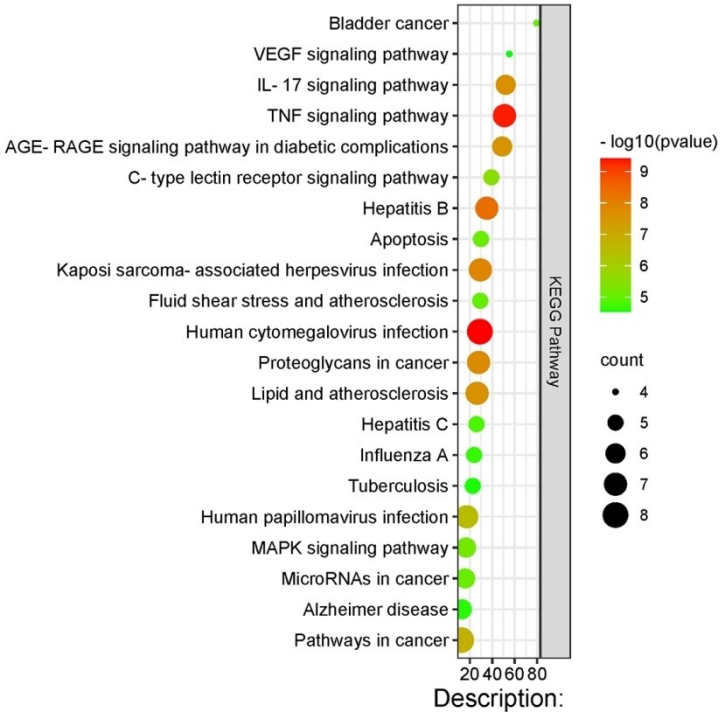

Figure 5. Bubble chart showing the relationship between the KEGG pathway and associated genes.

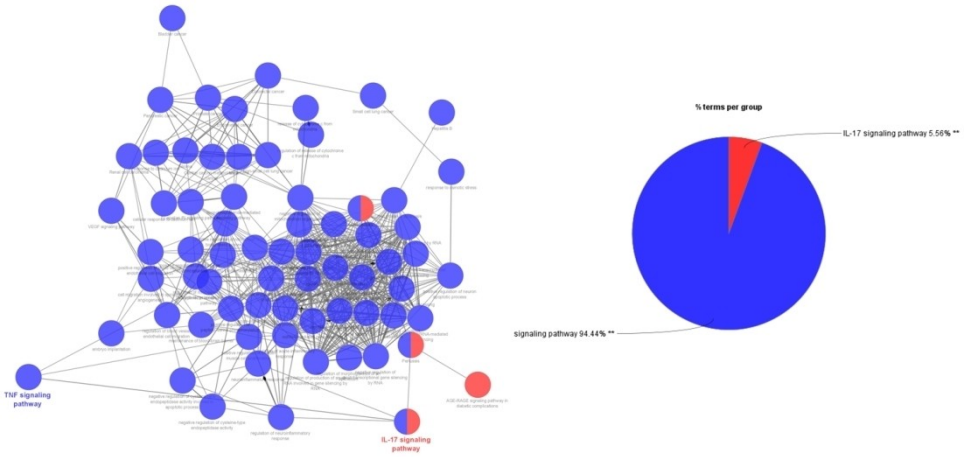

Figure 6. Clustering analysis of signaling pathways. Paths are indicated by circles. Main clusters are indicated in bold font.

receptor proteins via hydrogen bonds, hydrophobic forces, and van der Waals forces. These findings suggested that silybin may effectively treat MI by targeting these three proteins (Figures 8, 9, 10–11).

To validate the precision of our docking method, the co-crystallized ligand was docked within the binding pocket of TNF-α, IL-6, and AKT1, and the docked pose was compared with the crystal structure pose by calculating the RMSD values (0.956, 0.810, and 0.861). Figure 12 shows that the docked pose almost overlapped completely with the experiential orientation of the selected targets, indicating that our docking method is valid; therefore, all docking scores obtained are correct.

Effect of silybin on serum and cardiac biochemical parameters in rats

Several antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, contribute to the prevention of oxidative stress in biological systems. This study revealed that SOD in rats injected with serum ISO showed a significantly decreased serum SOD level (9.7 ± 0.32 to 3.3 ± 0.31; $p < 0.0001$***), heart SOD decreased (9.1 ± 1.37 to 4.1 ± 0.38; $P < 0.0001$***), serum catalase decreased (0.0021 ± 0.0002 to 0.001 ± 0.0002; $p < 0.0001$***), heart catalase decreased (0.00453 ± 0.00026 to 0.0011 ± 0.00029; $p < 0.001$**), serum LDH increased (93.2 ±

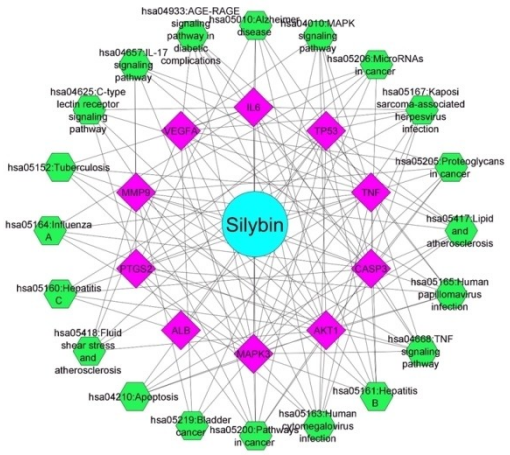

Figure 7. Silybin-target-signal pathway network.

8.04 to 227.5 ± 29; $p < 0.001$**), heart LDH increased (710.5 ± 148.64 to 2325.8 ± 169.98; $p < 0.0001$***), serum CkMB increased (88.4 ± 3.7 to 774 ± 82.46; $P < 0.0001$), and heart CkMB increased (622.6 ± 29.39 to 4612.1 ± 745.54; $p < 0.0001$***) compared to normal rats.

Isoprenaline-injected rats that received silybin treatment showed increased serum SOD increased (8.9 ± 0.42; $p < 0.0001$***), which provides evidence that silybin may modulate SOD activity. Similarly, serum catalase increased (0.0016 ± 0.0001; $p < 0.001$), Serum LDH decreased (97.2 ± 2.62; $P < 0.01$*), and serum CkMB decreased (125.1 ± 16.88; $p < 0.0001$***) in ISO rats. Similarly, heart SOD increased (0.00423 ± 0.00155; $p < 0.0001$***), heart CAT increased (0.00423 ± 0.00155; $p < 0.001$**), heart LDH decreased (802.2 ± 63.66; $p < 0.0001$***), heart CkMB decreased (29.17 ± 1.014; $p < 0.0001$***). Serum and cardiac biomarkers of myocardial injury, including CK-MB and LDH, are the key diagnostic features of MI. In line with this, increases in the levels of CK-MB and LDH were recorded in the ISO group compared to normal rats, which was expected after MI. However, the above parameters were maintained in ISO rats compared to ISO rats (Figures 13 and 14).

Isoproterenol (ISO), a chemically synthesized catecholamine, is an essential regulator of myocardial contractility and metabolism. Catecholamines offer broader therapeutic applications in maintaining blood pressure and normal cardiac function. However, excess oxidative catecholamine metabolism produces quinines that react with oxygen to produce ROS. ROS further lead to changes in myocardial structure, function, and biochemical parameters. Previous studies have provided significant insights into the critical role of lipid peroxidation and antioxidant enzyme status in the pathogenesis of ISO-induced myocardial infarction.

Similarly, our present study demonstrated a marked decline in the activity of the endogenous antioxidant enzyme SOD in ISO-treated rats. It contributes to increased ROS generation and decreased activity of the antioxidant defence system.[3] Phenolic compounds have been suggested to exert potent antioxidant effect.[1] In this respect, our study showed improved antioxidant defense in silybin-treated rats, with increased levels of SOD and GSH. Silybin is a flavonolignan extracted from milk thistle that exhibits an antioxidant effect by donating one hydrogen atom from its phenolic hydroxyl group. It effectively scavenges free radicals and prevents free radical-induced damage to the cell membrane. Phenolic compounds have been suggested to exert potent antioxidant effect.[2] In this respect, our study demonstrated improved antioxidant defense in silybin-treated rats with increased levels of SOD and catalase. The effect of silybin on the cardiac ECG revealed that the increased number of QRS complexes and decreased QRS-QRS duration ISO-induced (65.5 ± 0.764; $p < 0.0001$***), when compared to normal rats (17.17 ± 0.477). At doses of silybin of 25 mg/kg, 50 mg/kg, and 100 mg/kg, treated ratsshowed a significantly reduced number of QRS complex (65.67 ± 0.760; 32 ± 0.365; 29.17 ± 1.014; $p < 0.0001$***) and increased duration of QRS-QRS compared with ISO-inducedrats.ECG is currently one of the main tests used to diagnose myocardial infarction. Among the parameters observed on the ECG that indicated myocardial infarction, abnormalities in the QRS complex in ISO rats were restored by silybin treatment (Figures 15A and B).

Histological studies

Histological studies have revealed that cells can lose their cell permeability barrier properties during MI, leading to inflammation and degradation. Treatment with silybin A (25, 50, and 100 mg/kg) restored myocardial cell contactcompared to that in isoproterenol-induced rats. The intact myocardial volume was higher in the silybin A 100 mg/kg group than in the silybin A 25 mg/kg and 50 mg/kg treatment groups. Most importantly, histological alterations, characterized by a remarkable degree of lesions, degeneration, disruption, nuclear enlargement, binucleation, and loss of cardiomyocytes, replaced by disorganized fibroblasts and a few macrophages, as well as edema observed in the ISO group, were similar to those reported by others in experimental models of MI. The absence of histological alterations in silybin-treated ISO rats can be attributed to the ability of silybin to abate cardiac histological alterations (Figure 16).

Discussion

MI falls under the category of coronary atherosclerotic heart disease (CHD). Myocardial ischemia and hypoxia occur when the coronary blood supply is inadequate to meet the demand of the myocardium, myocardial ischemia, and hypoxia occurs. Angina pectoris can be induced by acute ischemia and hypoxia, whereas chronic ischemia and hypoxia can cause serious health effects, such as myocardial infarction.[8] The incidence of coronary heart disease and its high mortality have been observed in recent years. MI is a significant complication of thrombolysis and coronary intervention in patients.[9,10] MI often leads to poor prognosis and threatens the lives of victims. Consequently, it is essential to develop secure and efficient strategies for preventing MI.

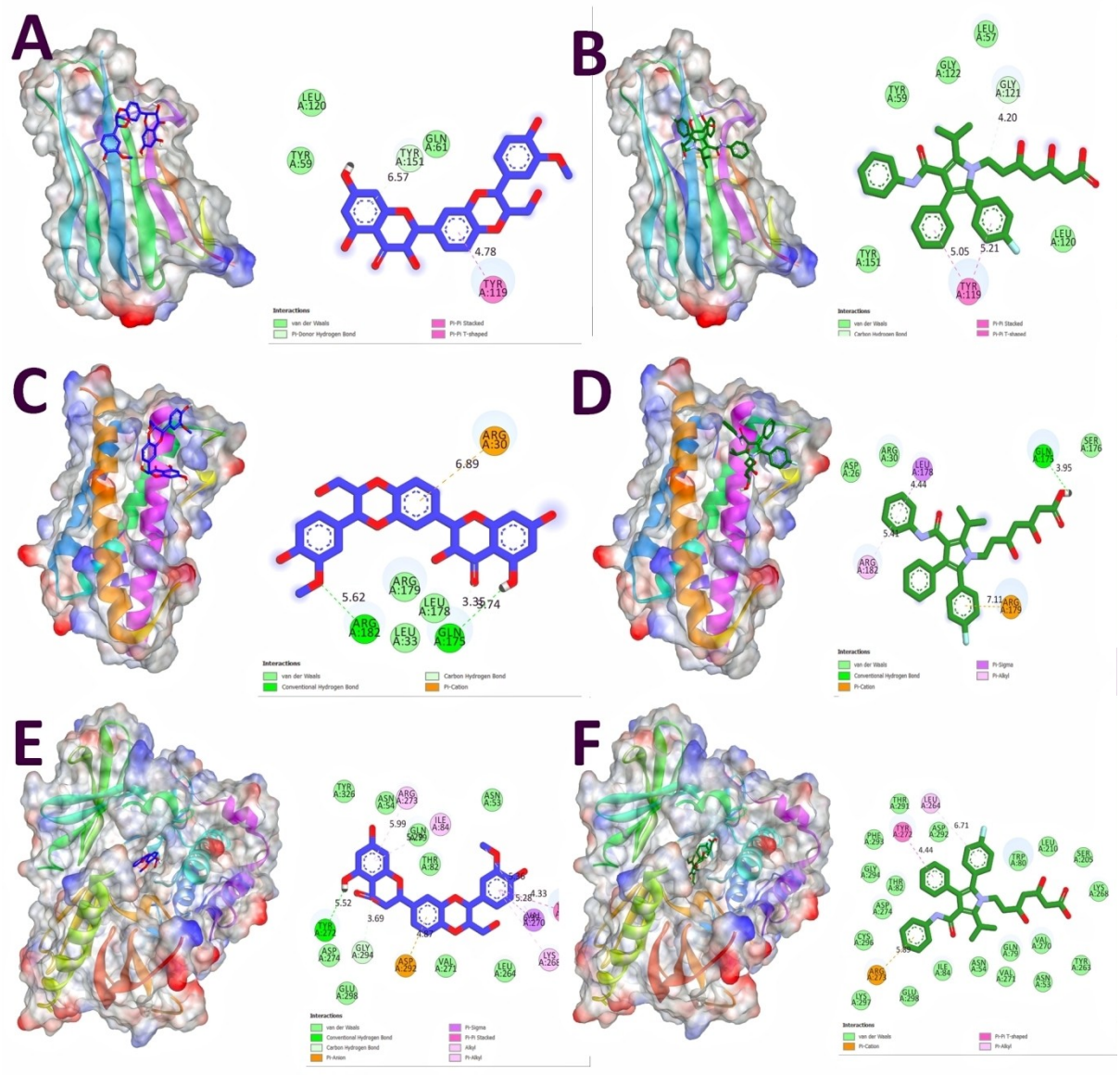

Figure 8. Molecular docking patterns of silybin and atorvastatin with various targets occupying the active pocket of proteins. (A) Silybin-TNF-α, (B) Atorvastatin-TNF-α, (C) Silybin-IL-6, (D) Atorvastatin-IL-6, (E) silybin AKT1, and (F) Atorvastatin-AKT1.

Owing to the lack of definitive information on how herbal drugs can be used to treat complex diseases, their development has become a significant challenge. Network pharmacology can be used to predict the interactions between multiple complex diseases and herbal interventions. Three phytochemicals, silymarin, silybin, silidianin, and silicristin, were derived from the milk thistle (Silybum marianum). This compound has a long history as a herbal remedy. Silybin is its main phytochemical and is widely credited with its health benefits.[11]

Silybin is usedmainly to treat metabolic disorders such as cardiovascular and cerebrovascular diseases. It can also protect the liver from hepatitis and prevent MI.[12,13] Scientists have determined that the mechanism of the clinical therapeutic effect of silybin varies with the deeper study of its pharmacology and molecular biology. Network pharmacology provides a method for understanding the pharmacological mechanisms underlying the multi-target effects of silybin. The MI treatment was based on 74 primary silybin targets. Gene enrichment analysis revealed that several biological processes, such as apoptosis, inflammatory response, glucocorticoids, and oxidative stress, were enriched. The signaling pathways most relevant to the significant targets were TNF-α, IL-17, C-type lectin receptor, MAPK, and VEGF.

Biochemical processes such as cell cycle control, metabolism, signalling pathways, and disease pathways are dependent on protein interactions. Systematic analysis of protein interactions is vital to interpret protein functions and better understand complex cellular processes.[14,15] The top 10 proteins calculated in degrees were obtained after the analysis of the PPI network. TNF-α, ALB, IL6, TP53, AKT1, VEGFA, MAPK3,

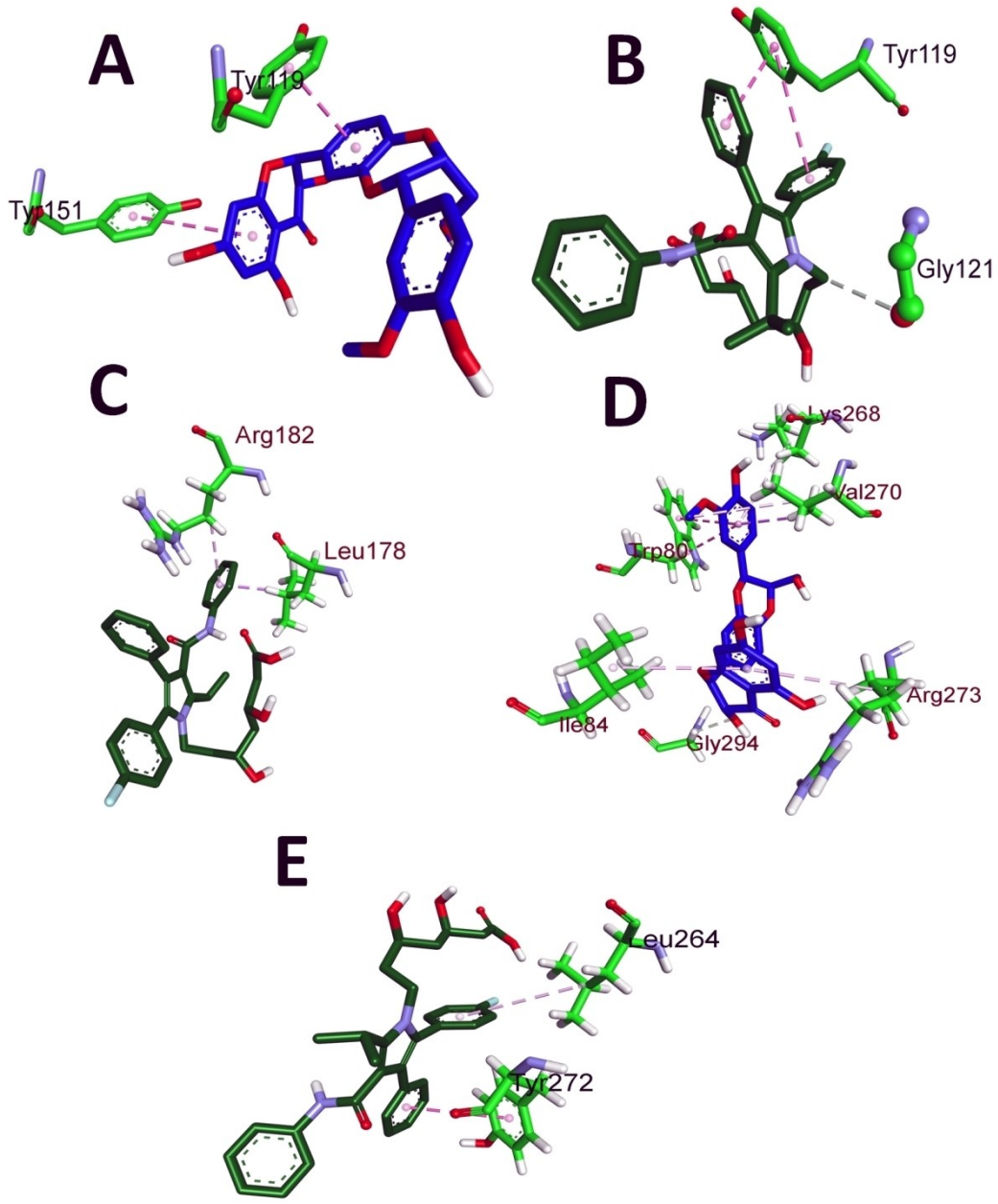

Figure 9. 3D Interaction of the ligands with the targets through hydrophobic interactions. (A) Silybin-TNF-α, (B) Atorvastatin-TNF-α, (C) Atorvastatin-IL-6, (D) Silybin-AKT1, and (E) Atorvastatin- AKT1.

PTGS2, MMP9, and CASP3.Based on the results obtained, the PPI network showed that AKT1, TNF-α, and IL-6 are critical targets of silybin in the treatment of MI.AKT1 significantly affects growth factor signal transduction, cell proliferation and differentiation, and transcriptional regulation and development. TNF stimulates angiogenesis in ischemic tissues. After myocardial infarction, the inflammatory factor TNF-α is highly expressed in the cardiac tissues. TNF-α promotes the expression and migration of bone marrow-derived mesenchymal stem cells (BMSC) from damaged human myocardium and their differentiation into cardiac cells, leading to improved heart function and repair.[16]

GO and KEGG pathway enrichment analyses showed that significant enrichment occurred in the TNF signaling pathway, IL-17 signalling pathway, MAPK signalling pathway, VEGF signalling pathway, HIF-1 signalling pathway, PI3K-Akt signalling pathway, lipid and atherosclerosis, fluid shear stress and, atherosclerosis and among others. PI3K/Akt modulates inflammatory cell activation, mediator release, and chronic inflammation.[17] HIF-1 stimulates genes that encode hypoxic homeostasis response proteins and increases glucose metabolism, cell proliferation, and angiogenesis, thus playing a vital role in ischemia and hypoxic myocardium.[18] ClueGO cluster analysis mainly characterized the TNF, IL-17, and VEGF signaling

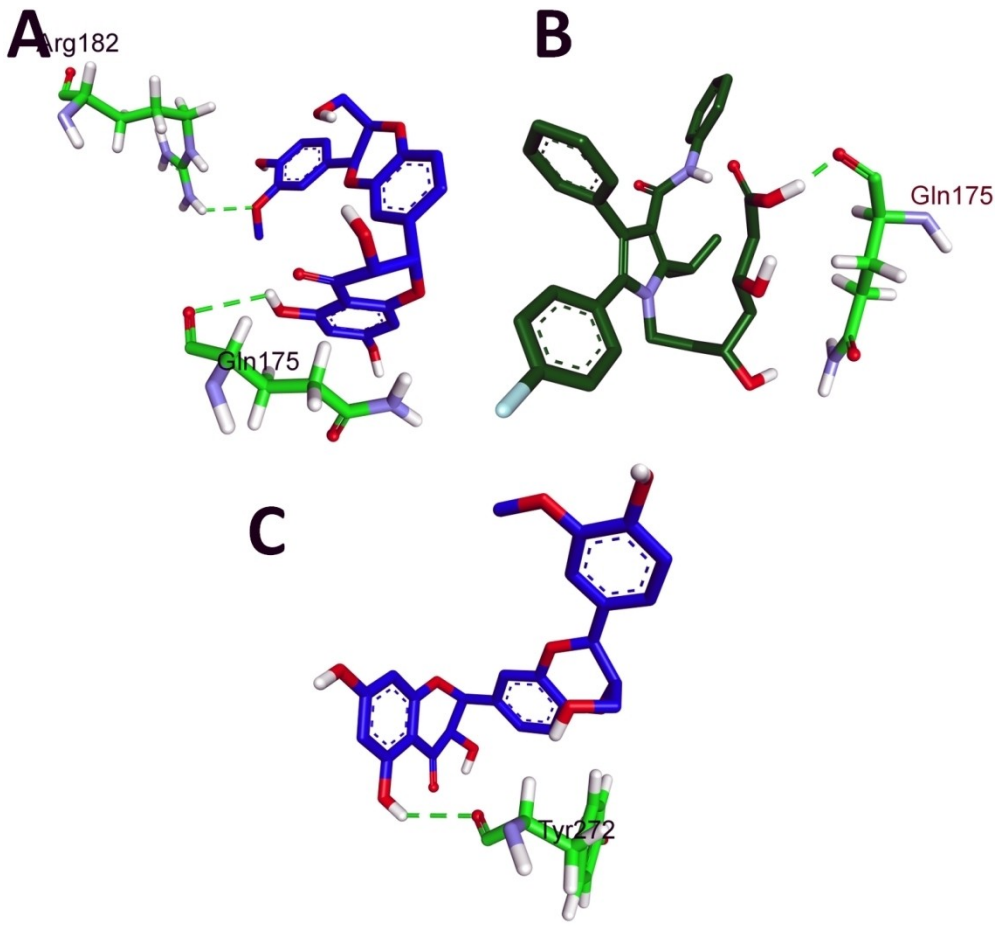

Figure 10. 3D Interaction of ligands with targets via hydrogen bond interactions. (A) Silybin -IL-6, (B) Atorvastatin-IL-6, and (C) Silybin-AKT1.

pathways. Studies suggest that IL17 stimulation leads to end-stage myocardial cell necrosis and loss of apoptosis.[19,20] Interestingly, apoptosis is strongly associated with the TNF, VEGF, and PI3K-Akt signaling pathways.[21,22] Extrinsic apoptotic pathways are mediated by TNF receptors, whereas hypoxia initiates intrinsic pathways.[23] The PI3K/AKT/HIF-1 signal transduction pathway causes mitochondrial dysfunction, leading to apoptosis and increased oxidative stress.[24] In this regard, current network pharmacology and *in silico* docking studies have reported that silybin targets PI3K/AKT/HIF-1 proteins, and our *in vivo* study showed that silybin-treated MI rats significantly improved SOD and catalase restored the QRS complex from the abnormal state, and the histopathology of the treated rats showed restored cell morphology compared to MI rats.

Conclusion

In conclusion, the present study demonstrated that silybin has a good regulatory effect on MI using echocardiography. Through network pharmacology, it was revealed that silybin mainly regulates TNF-α, IL6 and AKT1 proteins involved in various pathways, including the PI3K-Akt signaling pathways, TNF signalling pathways, VEGF signalling pathway and HIF-1 signalling pathways, and exerts an anti-MI effect. This laid the foundation for the mechanism and pharmacodynamic effects of silybin in the treatment of MI.

Supporting Information Summary

This section contains the Materials and methods Section and Tables (Table S1 to S5). Table S1: Common genes between silybin and MI, Table S2: Top 10 Hub gene identification by cytohubba and MCODE algorithm in the Cytoscape, Table S3: Gene ontology (GO) enrichmentin the top 10 Hub genes, Table S4: Target genes in the enrichment of signaling pathways related to TY2DM, Table S5: Binding energy and interactions of silybin on TNF-α, IL-6, and AKT1. Additional references cited within the Supporting Information.[25–48]

Funding

This study did not receive any external funding.

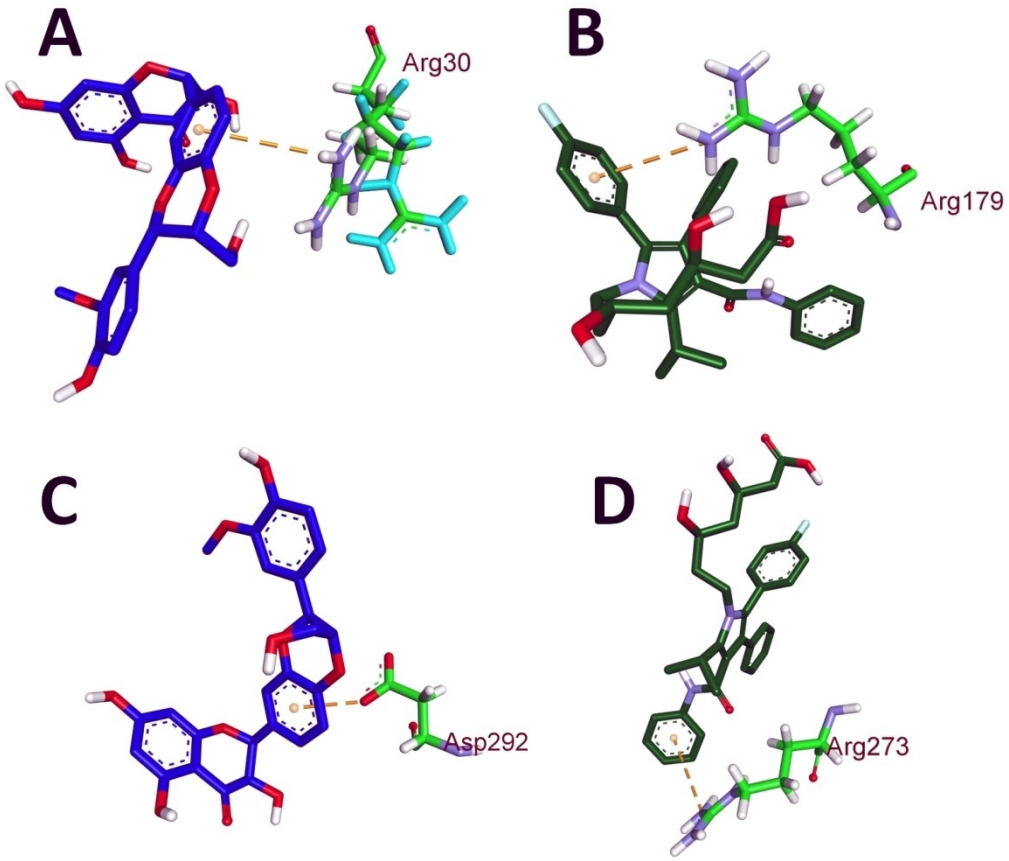

Figure 11. 3D Interaction of the ligands with the targets via electrostatic interactions. (A) Silybin-IL-6, (B) Atorvastatin-IL-6, (C) Silybin-AKT1, and (D) Atorvastatin-AKT1.

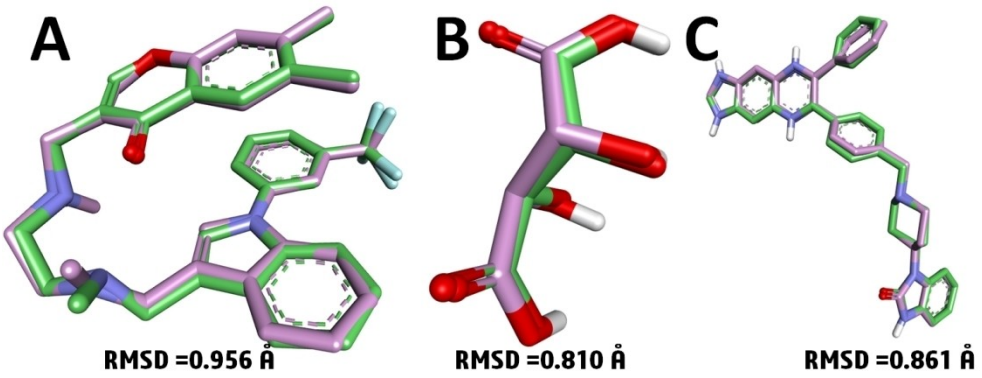

Figure 12. Validation of the molecular docking protocol employed using the before (green) and after docking (purple) poses of co-crystallized ligands. (A) 6,7-Dimethyl-3-[(methyl {2-[methyl({1-[3-(trifluoromethyl)phenyl]-1h-Indol-3-Yl}methyl)amino]ethyl}amino)methyl] 4h-chromen-4-one of TNF-α (PDB: 2AZ5), (B) L-(+)-Tartaric Acid of IL-6 (PDB: 1ALU), and (C) 1-(1-(4-(7-phenyl-1H-imidazo[4,5-g]quinoxalin-6-yl)benzyl)piperidin-4-yl)-1H-benzo[d]imidazol-2(3H)-one of AKT1 (PDB: 3O96).

Conflict of Interests

There is no conflict of interest with other people or organizations that could inappropriately influence or bias the content of the paper.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

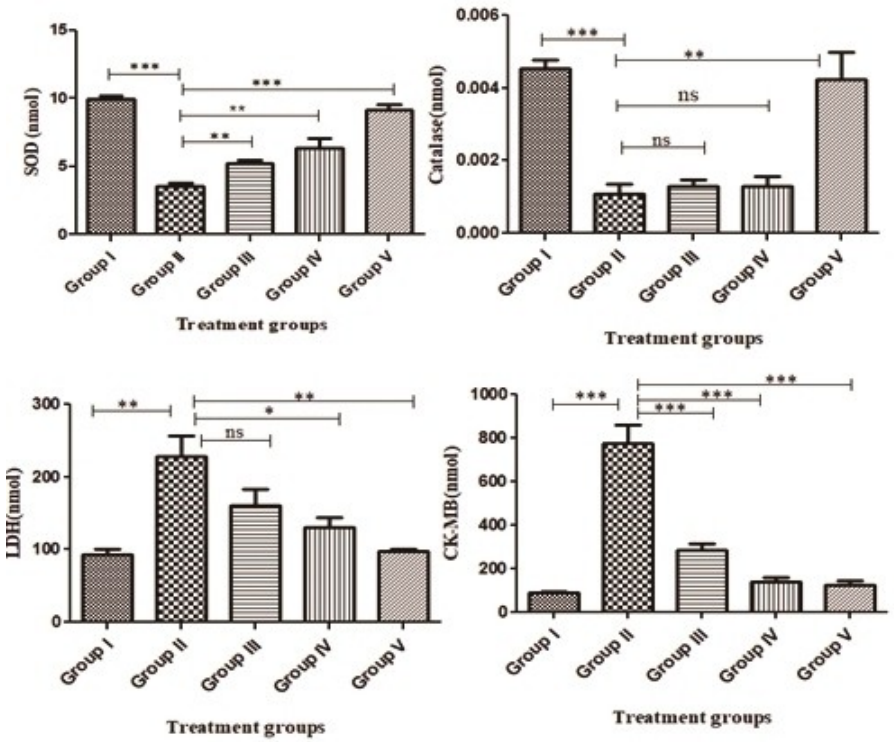

Figure 13. Effect of silybin on serum biochemical parameters in rats with isoproterenol-induced MI.

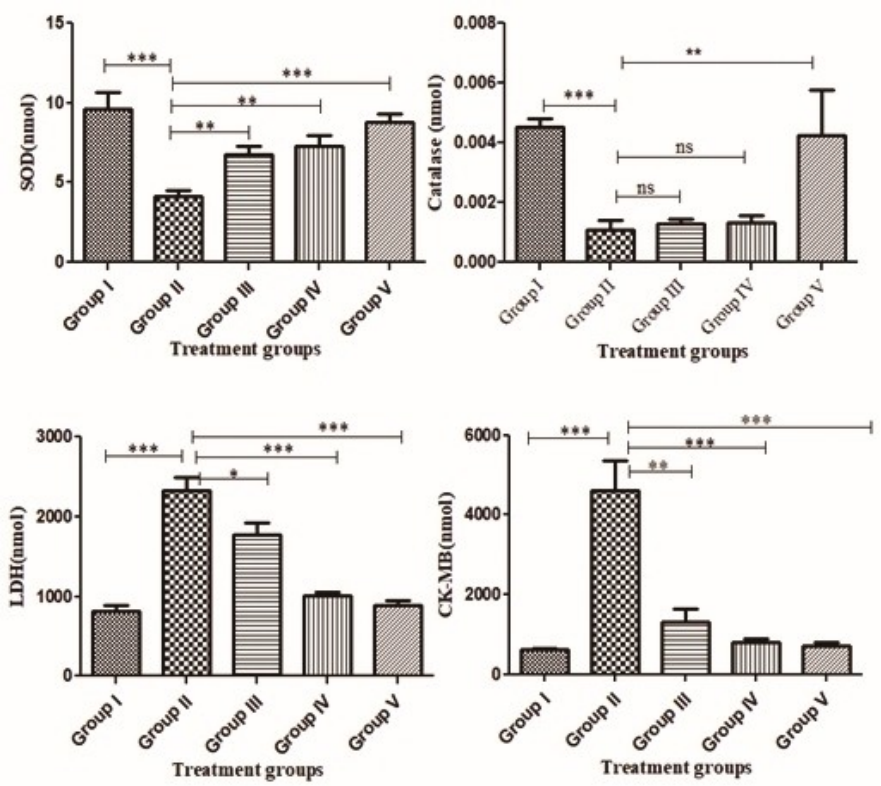

Figure 14. Effect of silybin on biochemical parameters ofheart tissue in isoproterenol-induced MI in rats.

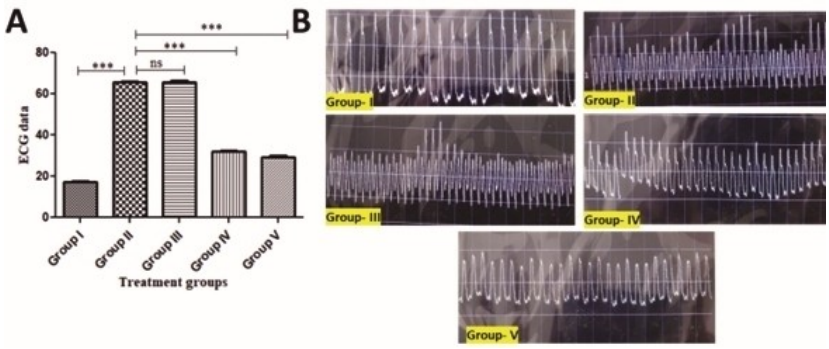

Figure 15. (A) Histogram of the effect of silybin on the number of QRS complex on isoproterenol-induced MI in rats; (B) Effect of silybin on the number of QRS complex compared to isoproterenol-induced MI in rats.

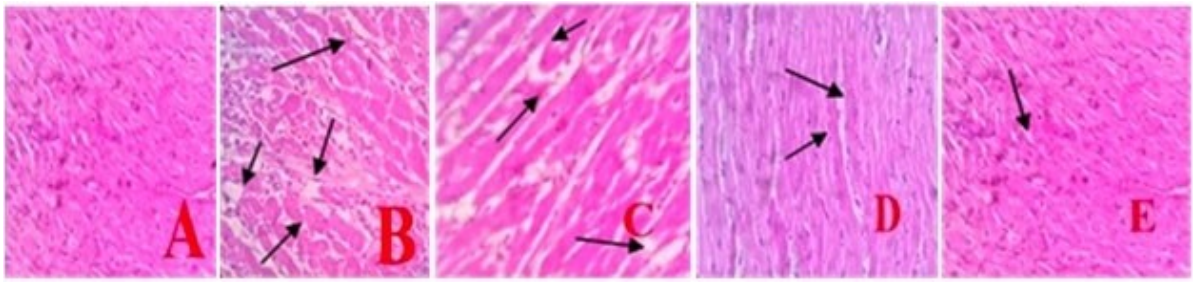

Figure 16. Myocardial tissue region (×40) after isoproterenol induction. (A) Normal rats, showingnormal cardiac muscle fibres; (B) ISO (100 mg/kg) control heart showing cardiac musclefibres with muscle separation and inflammatory cells; (C) Silybin treatment (25 mg/kg p.o.) + ISO (5 mg/kg s.c.); (D) silybin treatment (50 mg/kg p.o.) + ISO(5 mg/kg s.c.); (E) silybin (100 mg/kg p.o.) ISO (5 mg/kg SC).

Keywords: Antioxidant · Cardioprotective activity · Molecular docking · Myocardial infarction Network pharmacology · Silybin

[1] J. Khan, P. K. Deb, S. Priya, K. D. Medina, R. Devi, S. G. Walode, M. Rudrapal, *Molecules* **2021**, *26*, 4021.
[2] M. Rudrapal, S. J. Khairnar, J. Khan, A. B. Dukhyil, M. A. Ansari, M. N. Alomary, F. M. Alshabrmi, S. Palai, P. K. Deb, R. Devi, *Front. Pharmacol.* **2022**, *13*, 806470.
[3] M. Rudrapal, S. Maji, S. K. Prajapati, P. Kesharwani, P. K. Deb, J. Khan, R. Mohamed Ismail, R. S. Kankate, R. K. Sahoo, S. J. Khairnar, A. R. Bendale, *Antioxidants* **2022**, *11*.■■■Dear author, please add the page number.■■■
[4] P. Pasala, S. Siva Reddy, N. Silvia, D. Reddy, A. Sampath, N. Dorababu, N. Sirisha Mulukuri, K. Sunil Kumar, S. Chandana, M. Chetty, *J. King Saud Univ. Sci.* **2022**, *34*, 101828.
[5] Y. Dong, L. Hao, K. Fang, X. X. Han, H. Yu, J. J. Zhang, L. J. Cai, T. Fan, W. D. Zhang, K. Pang, W. M. Ma, X. T. Wang, C. H. Han, *BMC complementary medicine and therapies* **2021**, *21*, 45.
[6] I. Celik, M. Rudrapal, P. K. Yadalam, S. Chinnam, T. M. Balaji, S. Varadarajan, J. Khan, S. Patil, S. G. Walode, D. V. Panke, *Polycycl. Arom. Com.* **2022**, 1–18■■■Dear author, please add the volume number.■■■.
[7] C. Piao, Q. Zhang, D. Jin, L. Wang, C. Tang, N. Zhang, F. Lian, X. Tong, *Evid. Based Complement. Alternat. Med.* **2020**, *2020*, 6754761.
[8] G. Heusch, P. Libby, B. Gersh, D. Yellon, M. Bohm, G. Lopaschuk, L. Opie, *Lancet* **2014**, *383*, 1933–1943.
[9] H. J. Chi, M. L. Chen, X. C. Yang, X. M. Lin, H. Sun, W. S. Zhao, D. Qi, J. L. Dong, J. Cai, *Curr. Drug Targets* **2017**, *18*, 1712–1721.
[10] D. J. Hausenloy, D. M. Yellon, *J. Clin. Invest.* **2013**, *123*, 92–100.
[11] N. Janel, C. Noll, in *Polyphenols in Human Health and Disease* (Eds.: R. R. Watson, V. R. Preedy, S. Zibadi), Academic Press, San Diego, **2014**, pp. 665–679.
[12] H. Chen, D. F. Su, T. H. Zhang, H. J. Ding, Y. C. Yang, S. C. Chen, *Zhongguo Yaoli Xuebao* **1992**, *13*, 69–71.
[13] A. Islam, A. Mishra, M. A. Siddiqui, S. Siddiquie, *Drug Res. (Stuttg)* **2021**, *71*, 489–503.
[14] S. K. Miryala, A. Anbarasu, S. Ramaiah, *Gene* **2018**, *642*, 84–94.
[15] N. Safari-Alighiarloo, M. Taghizadeh, M. Rezaei-Tavirani, B. Goliaei, A. A. Peyvandi, *Gastroenterol. Hepatol. Bed. Bench.* **2014**, *7*, 17–31.
[16] M. R. Shin, S. K. Kang, Y. S. Kim, S. Y. Lee, S. C. Hong, E. C. Kim, *Int. Endod. J.* **2015**, *48*, 705–716.
[17] H. Jiang, P. W. Abel, M. L. Toews, C. Deng, T. B. Casale, Y. Xie, Y. Tu, *J. Pharmacol. Exp. Ther.* **2010**, *334*, 703–709.
[18] L. J. Ambrose, A. H. Abd-Jamil, R. S. Gomes, E. E. Carter, C. A. Carr, K. Clarke, L. C. Heather, *J. Cardiovasc. Pharmacol. Ther.* **2014**, *19*, 574–585.
[19] Y. Ren, R. Wang, L. Jiang, X. Wang, Z. Feng, J. He, X. He, G. Du, *Ann. Clin. Lab. Sci.* **2020**, *50*, 761–768.
[20] J. L. Su, Y. R. Jiao, T. Li, H. C. Yao, *Int. J. Cardiol.* **2017**, *239*, 29.
[21] L. Ou, S. Lin, B. Song, J. Liu, R. Lai, L. Shao, *Int. J. Nanomed.* **2017**, *12*, 6633–6646.
[22] A. A. Shati, *Syst. Biol. Reprod. Med.* **2019**, *65*, 236–249.
[23] E. Teringova, P. Tousek, *J. Transl. Med.* **2017**, *15*, 87.
[24] L. Yiming, H. Yanfei, Y. Hang, C. Yimei, S. Guangliang, L. Shu, *Toxicology* **2021**, *451*, 152694.
[25] A. Daina, O. Michielin, V. Zoete, *Nucleic Acids Res.* **2019**, *47*, W357–W364.
[26] G. Stelzer, N. Rosen, I. Plaschkes, S. Zimmerman, M. Twik, S. Fishilevich, T. I. Stein, R. Nudel, I. Lieder, Y. Mazor, S. Kaplan, D. Dahary, D. Warshawsky, Y. Guan-Golan, A. Kohn, N. Rappaport, M. Safran, D. Lancet, *Curr. Protoc. Bioinforma.* **2016**, *54*, 13031–313033.
[27] D. S. Wishart, C. Knox, A. C. Guo, S. Shrivastava, M. Hassanali, P. Stothard, Z. Chang, J. Woolsey, *Nucleic Acids Res.* **2006**, *34*, D668–672.
[28] J. Pinero, N. Queralt-Rosinach, A. Bravo, J. Deu-Pons, A. Bauer-Mehren, M. Baron, F. Sanz, L. I. Furlong, *Database (Oxford)* **2015**, *2015*, bav028.
[29] J. S. Amberger, C. A. Bocchini, A. F. Scott, A. Hamosh, *Nucleic Acids Res.* **2019**, *47*, D1038–D1043.

[30] C. H. Chin, S. H. Chen, H. H. Wu, C. W. Ho, M. T. Ko, C. Y. Lin, *BMC Syst. Biol.* **2014**, *8 Suppl 4*, S11.
[31] C. The Gene Ontology, *Nucleic Acids Res.* **2017**, *45*, D331–D338.
[32] M. Kanehisa, M. Furumichi, Y. Sato, M. Ishiguro-Watanabe, M. Tanabe, *Nucleic Acids Res.* **2021**, *49*, D545–D551.
[33] W. Huang da, B. T. Sherman, R. A. Lempicki, *Nat. Protoc.* **2009**, *4*, 44–57.
[34] S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B. A. Shoemaker, P. A. Thiessen, B. Yu, L. Zaslavsky, J. Zhang, E. E. Bolton, *Nucleic Acids Res.* **2019**, *47*, D1102–D1109.
[35] D. S. Goodsell, C. Zardecki, L. Di Costanzo, J. M. Duarte, B. P. Hudson, I. Persikova, J. Segura, C. Shao, M. Voigt, J. D. Westbrook, J. Y. Young, S. K. Burley, *Protein Sci.* **2020**, *29*, 52–65.
[36] P. K. Pasala, R. Abbas Shaik, M. Rudrapal, J. Khan, M. A. Alaidarous, S. Jagdish Khairnar, A. R. Bendale, V. D. Naphade, R. Kumar Sahoo, J. H. Zothantluanga, S. G. Walode, *Saudi J. Biol. Sci.* **2022**, *29*, 998–1005.
[37] J. H. Zothantluanga, M. Abdalla, M. Rudrapal, Q. Tian, D. Chetia, J. Li, *Polycycl. Arom. Comp.* **2022**, doi: 10.1080/10406638.2022.2046613.
[38] M. Rudrapal, I. Celik, S. Chinnam, M. A. Ansari, J. Khan, S. Alghamdi, M. Almehmadi, J. H. Zothantluanga, S. J. Khairnar, *Saudi J. Biol. Sci.* **2022**, *29*, 3456–3465.
[39] A. E. James, U. C. Okoro, M. A. Ezeokonkwo, C. R. Bhimapaka, M. Rudrapal, D. I. Ugwu, N. Gogoi, D. Chetia, I. Celik I, *ChemistrySelect* **2022**, *7*, e202103908.
[40] M. Rudrapal, I. Celik, J. Khan, R. M. Ismail, M. A. Ansari, R. Yadav, T. Sharma, T. E. Tallei, P. K. Pasala, R. K. Sahoo, S. J. Khairnar, A. R. Bendale, J. H. Zothantluanga, D. Chetia, S. G. Walode, *J. King Saud Univ. Sci.* **2022**; *34*, 101826.
[41] M. Rudrapal, N. Gogoi, D. Chetia, J. Khan, S. Banwas, B. Alshehri, M. A. Alaidarous, U. D. Laddha, S. J. Khairnar, S. G. Walode, *Saudi J. Biol. Sci.* **2022**, *29*, 2432–2446.
[42] J. A. Junejo, K. Zaman, M. Rudrapal, I. Celik, E. I. Attah, *South African J. Bot.* **2021**, *143*, 164–173.
[43] M. Rudrapal, I. A. Rashid, C. Agoni, A. R. Bendale, A. Nagar, M. E. S. Soliman, D. Lokwani, *J. Biomol. Struct. Dyn.* **2022**, *40*, 10437–10453.
[44] I. M. M. Othman, M. H. Mahross, M. A. M. Gad-Elkareem, M. Rudrapal, N. Gogoi, D. Chetia, K. Aouadi, M. Snoussi, A. Kadri, *J. Mol. Struct.* **2021**, *1243*, 130862.
[45] I. Celik, M. Rudrapal, P. K. Yadalam, S. Chinnam, T. M. Balaji, S. Varadarajan, J. Khan, S. Patil, S. G. Walode, D. V. Panke, *Polycycl. Arom. Comp.* **2022**, doi: 10.1080/10406638.2022.2091618.
[46] A. K. Umar, J. H. Zothantluanga, S. K. Aswin, S. Maulana, M. S. Zubair, H. Lalhlenmawia, M. Rudrapal, D. Chetia, *Sruct. Chem.* **2022**, doi: 10.1007/s11224-022-01959-3.
[47] J. Devasia, S. Chinnam, K. Khatana, S. Shakya, F. Joy, M. Rudrapal, A. Nizam, *Polycycl. Arom. Comp.* **2022**, doi: 10.1080/10406638.2022.2036778.
[48] P. K. Pasala, R. K. Uppara, M. Rudrapal, J. H. Zothantluanga, A. K. Umar, *J. Biochem. Mol. Toxicol.* **2022**, *36*, e23072.

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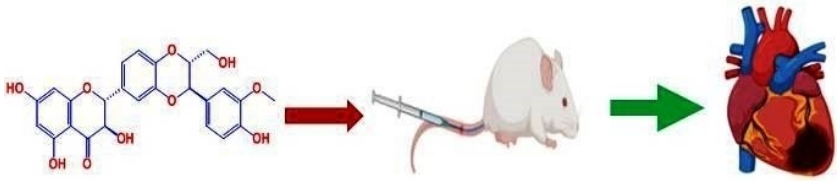
RESEARCH ARTICLE



This study suggests that silybin alleviates myocardial infarction (MI) by improving antioxidant levels through the PI3K-Akt/HIF-1 pathway from network pharmacology, molecular docking, and *in vivo* Studies.

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