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Integrating The Network Pharmacology and Molecular Docking to Uncover The Potential Mechanism Of Rutin In Fighting Diabetes Mellitus

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ABSTRACT

Introduction*: Rutin is a flavonol glycoside that is known to have blood sugar reducing activity. However, its molecular mechanism in reducing blood sugar level remains unclear. This study was employed to elucidate the pharmacological mechanism of rutin as antidiabetic agent.* ***Methods****: Potential target of rutin was screened in relevant databases to construct a compound-target network. Network pharmacology was utilized to identify targets associated with disease, gene ontology and KEGG pathways and confirmed its potential binding affinity using Autodock 4.2 assisted by ADT interface* ***Result:*** *. The result highlighted mTor, PIK3R1, and NFKB1R as a potential target of Rutin through network pharmacology. This target involved in the insulin signaling pathways, insulin resistance, type 2 diabetes mellitus, B receptor signaling pathways, AGE-RAGE signaling pathway in diabetic complications and pancreatic cancer. All docking protocols were valid with RMSD value for TNF-a, NF-KB, PI3K were 0.72 Å, 0.67 Å, and 0.54 Å, respectively. The molecular docking has confirmed the potential mechanism of rutin as antidiabetic agent by stably bound with these proteins with estimated free binding energy values of -8.54 kcal/mol (NF-KB), -8.01 kcal/mol (PI3K), and -6.22 kcal/mol (TNF-α).* ***Conclusion:*** *The study has given insight into the molecular mechanism of rutin in the management of DM by stably bound with NF-KB, TNF-α, and PI3K. However, further laboratory experimental research is needed, particularly in vitro and in vivo assay.*

KEYWORDS*: Rutin, antidiabetic, network pharmacology, molecular docking, virtual screening*

INTRODUCTION

Herbal medications are expending our perspectives and becoming more viable options for treating a range of disease (Madkour et al., 2024). Herbal medication is potentially good source for screening α-glucosidase inhibitors. Flavonoid and phenolic compounds, widely present in various plants inhibit α-glucosidase. Rutin are commonly used as standard compound for total phenolic and/or total flavonoid measurement (Limanto et al., 2019). The molecular structure of Rutin consist of 3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside (Figure1).

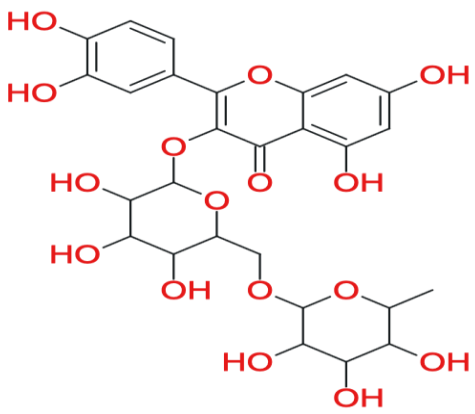


Figure 1. Two-dimensional structure of rutin

Flavonol rutin is widely distributed in plant, including buckwheat seed, berries, tea, and pagoda tree blossom. Chemically, it is a glycoside made up of the disaccharide rhamnosyl glucose and the flavonol aglycone quercetin (Choi et al., 2021). Rutin and quercetin were reported to possess anti-diabetic potentials. Some studies proposed that rutin and quercetin exert its hypoglycemic effect through multiple actions including by increasing the proliferation of pancreatic β-cell, enhancing insulin sensitivity and stimulating insulin secretion. In addition, rutin and quercetin were shown to strongly inhibit α-glucosidase (Limanto et al., 2019).

Rutin has garnered attention for its potential antidiabetic effects. Its multifaceted approach to combating diabetes and its complications makes it a promising subject for further research and potential therapeutic applications. The mechanisms by which rutin exerts its antidiabetic effects are diverse and target multiple aspects of glucose metabolism and diabetes pathology including limiting carbohydrate absorption in the small intestine, suppressing gluconeogenesis in tissues, enhancing glucose uptake, promoting insulin secretion from beta cells, and safeguarding the kidneys from deterioration. Furthermore, rutin diminishes the production of sorbitol, reactive oxygen species, advanced glycation end product precursors, and inflammatory cytokines. The study's findings suggest that rutin could be beneficial in preventing or managing diabetes-related conditions. (Ghorbani, 2017). However, its cellular and molecular mechanism remains unclear.

One of the diseases posing a global threat to public health is diabetes mellitus (DM). The World Health Organization estimates that by 2030, DM would rank as the sixth most common cause of death globally due to its rapidly rising prevalence. A metabolic condition known as diabetes mellitus is typified by elevated blood glucose levels brought on by deficiencies in the action, secretion, or both of insulin (insufficient or inefficient insulin) (Al-Ishaq et al., 2019).

The complexity of human diseases, often rooted in multiple gene groupings, presents a significant challenge in modern medicine. This genetic intricacy necessitates sophisticated approaches to drug discovery and development. However, the traditional drug development process is notoriously expensive and time-consuming, as noted by Firzannida et al. (2022). This inefficiency has spurred researchers to seek innovative methods to streamline and enhance the drug discovery pipeline Network pharmacology emerges as a potential approach to guide drug discovery.

This systematic research method combines data analysis with experimental and clinical studies to inform and steer the drug discovery and development process (Han et al., 2022). Network pharmacology primary focus is recognize and evaluate the complex interaction that are present between compund, target, and disease in biological system. This method makes it possible to completly understand how the therapeutic process functions and how it affects biological networks rather than simply spesific targets or pathways. Large-scale biological and pharmacological data identification and analysis is carried out through the use of computational and systems biology technologies in network pharmacology (Ihya et al., 2024).

One crucial computational technique within the network pharmacology toolkit is molecular docking. As explained by Frimayanti et al. (2021), molecular docking is a method used to predict chemical bonds between macromolecules (receptors) and small molecules (ligands). This prediction is based on their structures and is carried out through molecular bond simulations. The primary goal of molecular docking is to determine the conformation and binding free energy involved in the interaction between the receptor and the ligand. The integration of molecular docking into the broader framework of network pharmacology represents a powerful synergy. While network pharmacology provides the overarching strategy for understanding complex biological interactions, molecular docking offers a detailed, atomistic view of how potential drug compounds might interact with their targets. This combination of broad-scale analysis and fine-grained prediction enhances researchers' ability to identify promising drug candidates and understand their potential effects within the body (Luthfiana et al., 2023).

This study aims to explore and comprehensively elucidate the potential mechanism of rutin as an antidiabetic agent through an integrated network pharmacology and molecular docking simulations. By using this technique, it is hoped that information can be obtained regarding the molecular pathways of rutin and its binding affinity to molecular targets that are relevant in the management of DM. It is hoped that the results of this research will provide a stronger scientific basis for the development of rutin as a therapeutic agent for diabetes mellitus.

MATERIAL AND METHODS

Network Pharmacology Analysis

Target Identification of Rutin

The simplified molecular-input line-entry specification (SMILES) information of rutin was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/), and subsequently submitted to Super-PRED (https://prediction.charite.de/) to predict the target of Rutin. The Homo sapiens mode was used in acquiring protein linked to rutin compound target. To standardize protein IDs and edfliminated duplicate protein, STRING

database (https://string-db.org/) was used for protein ID alignment. Platform used to collect target related to Diabetic mellitus are the GeneCards (https://www.genecards.org/) databases, while the VENNY 2.1 tool (https://bioinfogp.cnb.csic.es/tools/venny/) identified separate and overlapping targets between diabetic mellitus and rutin.

Construction of protein-protein interaction (PPI)

All of the protein that came from the previous step was entered into the STRING database (https://string-db.org/) using the "multiple protein" option. The parameter setting included Homo sapiens as the organism, "full STRING network" as type of network, a "high confidence" score (0.400), and an FDR stringency of medium (5 percent)". PPI data in TSV format was downloaded from the "explore" option and then analyzed using Cytoscape 3.10.2 software to visualize and construct the PPI network.

Network construction and topological analysis using Cytoscape software

The TSV format of PPI data was imported into Cytoscape 3.10.2 software for topological analysis. A network topology parameter analysis was carried out using the cytoscape network analyzer, with nodes depicting target, pathways and compound. The interactions between node representatives are displayed on the edges. The influence increases with the number of nodes that are directly connected to each other.

GO and KEGG Enrichment Analysis

To determine the target role in signaling pathways, the ShinyGO 0.80 tool (http://bioinformatics.sdstate.edu/go/) was used to interpret GO biological process, GO celullar component, GO molecular function and KEGG pathway of intersecting targets. The parameter was set at FDR < 0.05, and the result were visualized as bar plot map.

Molecular Docking Studies

Ligand Preparation

The Test compounds used for virtual screening were obtained from literature studies. The 3D conformations of rutin target compounds were downloaded from PubChem (PubChem (nih.gov)).

Protein Structure Preparation

The receptors used in this study were NF-kB, TNF-α, PI3K, in which the structure of these target proteins was obtained from Protein Data Bank (PDB) (rcsb.org) with PDB IDs were 4D5N, 4JPS, and 2AZ5, respectively. The proteins were selected based on several criteria including mutation (nil), resolution (≤2.5 Å) capturing method (X-ray diffraction). The proteins downloaded from PDB were then prepared using BIOVA Discovery Studio software. In the preparation of proteins, water molecules, ligands and other heteroatoms were removed from the protein structures. On the other hand, polar hydrogen atoms were added to the protein structures.

Table 1. Adjustment of coordinate and size of the gridbox

Macromolecules	Coordinate			Size		
	Center X	Center Y	Center Y	Size X	Size Y	Size Z
TNF-α	-19.163	74.452	33.837	40	40	40
PI3K	-1.166	-9.035	16.981	40	40	40
NF-KB	-8.845	29.620	-3.895	40	40	40

Table 2. Target genes in KEGG signaling pathways enrichment related to DM

Pathway	No of genes	False Discovery Rate (FDR)	Genes
Insulin resistance	6	1.9×10^{-8}	MTOR, NFKB1, PIK3R1, PTPN11, RPS6KA3, SLC2A1.
Type II diabetes mellitus	2	1.42×10^{13}	MTOR, PIK3R1.
AGE-RAGE signaling pathway in diabetic complications	2	4.98×10^{14}	NFKB1, PIK3R1.
Insulin signaling pathway	2	8.08×10^{14}	MTOR, PIK3R1.
Pancreatic cancer	3	1.19×10^{14}	MTOR, PIK3RI, NFKB1,
B cell receptor signaling pathway	2	3.54×10^{14}	NFKB1, PIK3RI.

Molecular Docking Study

The molecular docking study was carried out by using AutoDock software assisted by AutoDockTools. The protein active site was determined following to the native ligand binding site of each protein. Molecular docking parameters were used according to the default value. The coordinate of the gridbox and adjustment of x, y and z can be seen in Table 1 with spacing of 0.375 Å. The Lamarckian Genetic Algorithm (LGA) was used in docking process with 100 runs of genetic algorithm parameter, default mutation rate and crossover rate. The molecular docking method are valid if the RMSD value obtained from the re-docking of native ligand is less than 2Å.

RESULTS AND DISCUSSION

Result

Network pharmacology analysis

Database retrieval of Rutin targets upon thesubmittings the SMILES code of Rutin to the database, it resulted in 97 targets (Super PRED). The targets are compiled and combined into an Excel format, which was the imported to STRING (https://string-db.org/) to eliminate duplicate protein targets and obtain valid protein ID names. The complete list of all targets is provided in Table 2. The Result of the retrieval of diabetic mellitus gene targets from related databases revealed the acquisition of 171 targets from GeneCards predictions (Figure 2). Cross-matching of these gene targets with

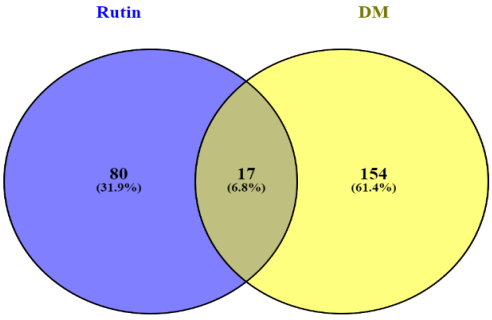


Figure 2. Overlapping genes of rutin related genes from DM.

the 97 targets from Super-PRED analysis identified 17 (6,8%) common targets in close and direct associated with diabetic mellitus and rutin compound (Figure 2).

Protein-protein interaction network.

Protein IDs resulting from the STRING database (https://string-db.org/). To visualize this interaction the outcomes were imported into Cytoscape 3.10.2 software for topological analysis. The finding of this analysis is presented in (Figure 3).

GO and KEGG enrichment analysis.

GO functional annotation and KEGG pathway analysis was performed on 17 targets of PPI network. The top 20 were the visualized as a bar plot chart (Figure 4). In the biological process, Rutin has great influence on response to nitrogen compound (Figure 4a) which means that rutin might alter the building block production. At the cellular component, the function of rutin is mainly related to cytoplasmic vesicle and intracellular vesicle (Figure 4b). Targets in the molecular function are closely related to insulin receptor binding, phosphoprotein binding, and enzyme binding (Figure 4c). A total of 6 KEGG signaling routes related to diabetic mellitus were discover at a threshold P-value of <0.05.

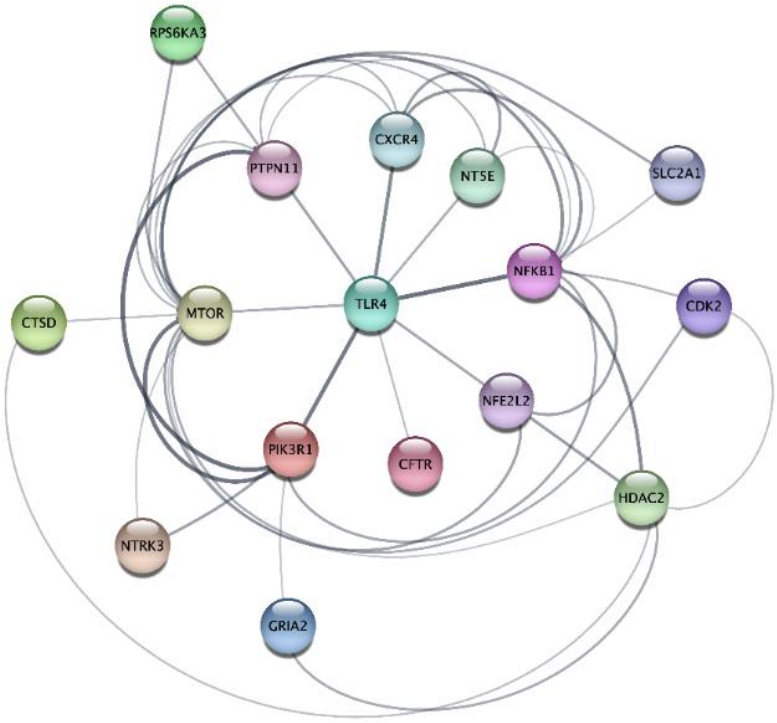


Figure 3**.** The visualization of PPI network from common genes

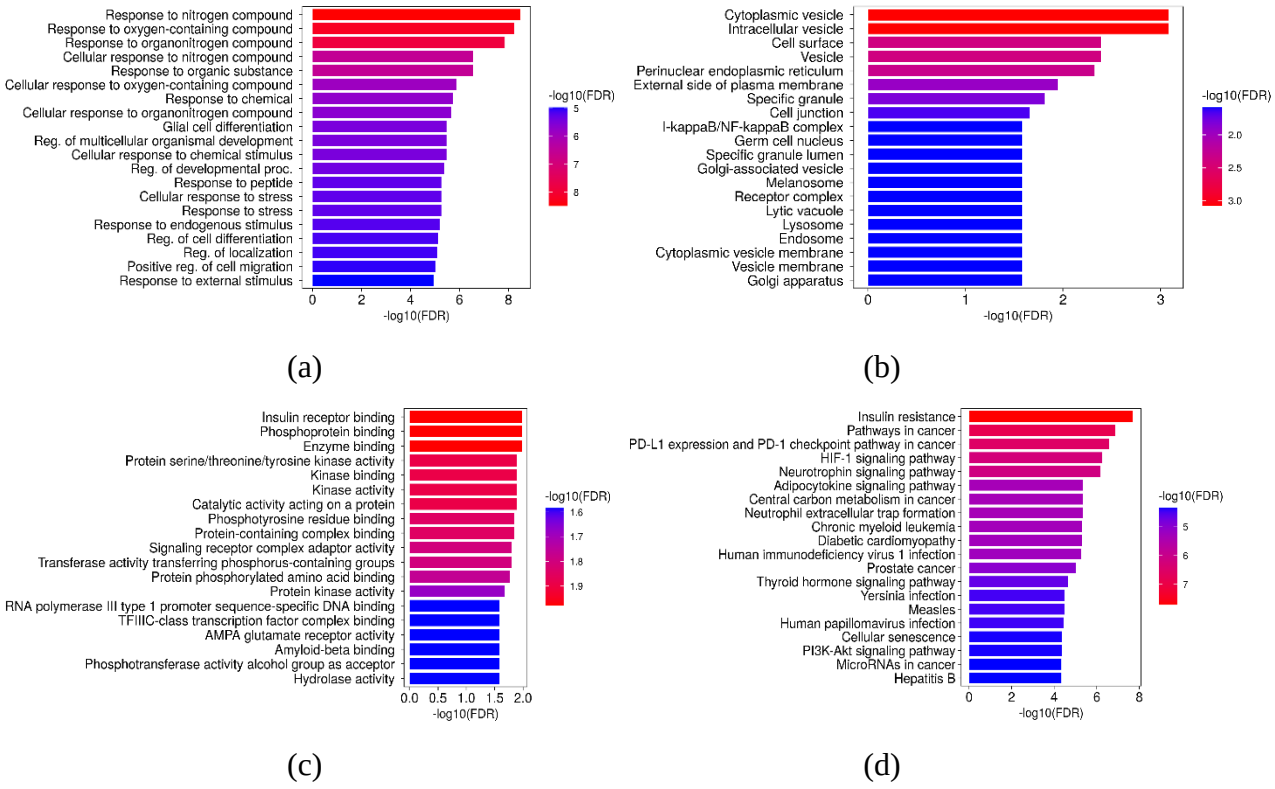


Figure 4. Enrichment analysis of potential from common target of Rutin and Diabetic mellitus:(a) GO Biological Process; (b) GO Cellular Component; (c) GO Molecular Function; (d) KEGG Pathways

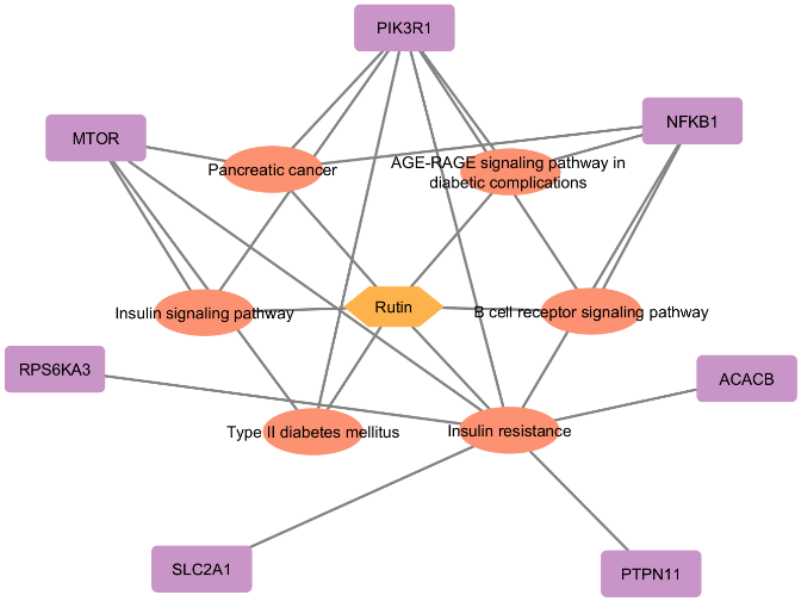


Figure 5. Network diagram of rutin-target-pathways

Thereafter, the FDR values were calculated which reveal the level of pathway enrichment with significantly low FDR values (Table 2 Figure 4d). On further analysis and using the score strength and count from the bar plot, the Insulin resistance pathway was identified as the best and most enriched route within the

assigned intersecting target genes with an FDR value of 1.9 x 10^{-8} (Table 2).

Network construction of compound-core target-pathways.

After obtaining the result of core target and pathways, a visualization of network construction was presented to understand the relationship between Rutin, its main target, and associated pathways using Cytoscape software 3.10.2 (Figure 5).

Molecular Docking

Ligand Selection

Molecular docking-based virtual screening

Molecular docking begins with validation of the docking method by simply re-docking to the target receptors (Figure 6). The RMSD values obtained from re-docking TNF-a, PI3K, and NF-KB were 0.72 Å, 0.67 Å, and 0.54 Å, respectively. These results indicate that the docking method used is valid due to the RMSD value were less than 2 Å. The specified limit suggests that the computationally predicted binding configurations align fairly well with the experimentally determined structures (Figure 6). This alignment takes into account the natural flexibility of proteins and the simplifications inherent in docking simulation methods (Utami et al., 2023). After validating the docking method, an evaluation of the target compounds was conducted. To obtain the best compounds, the screening results of the target compounds obtained from molecular docking were further analyzed by observing the lowest binding energy values. Test compounds with lower binding energy values than the original ligand indicated better binding strength to the receptor. Additionally, analysis is conducted by observing the interactions between the ligand and the amino acid residues on the receptor. The binding energy values of the test compounds and original ligands can be seen in Table 3.

Table 3. The docking scores of rutin

Test Compounds	Receptors/binding energy(kcal/mol)		
	TNF-α	PI3K	NF-KB
Rutin	-6.22	-8.01	-8.54

The result in Table 3 showed that the test compounds have different binding affinity on each receptor. Although there are several compounds that have potential for more than one receptor, several other compounds work specifically on one particular receptor. Rutin was fit in the NF-KB, PI3K, and TNF-α binding site and interacted with various

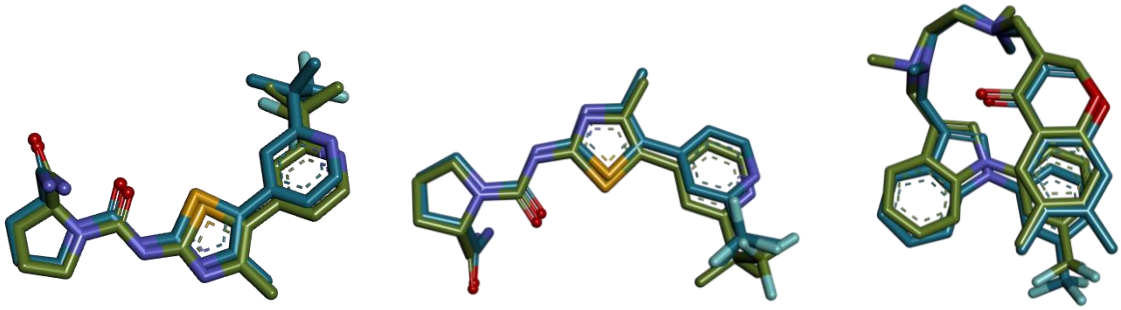
Table 4. Summarize of essential amino acid residues of target macromolecules interacted with rutin

Macromolecules	Hydrogen interactions	Hydrophobic interactions
NF-KB	Asf 515, Ser 371, Ser 410, Gln 479, Ser 476, Asf 534, Lys 429, Gly 407, Arg 408	Leu 406, Val 414, Leu 522, Cys 533, Asf 519
PI3K	Val 851, Ser 854, Glu 849, Gln 859, Tyr 836, Lys 802, Asp 933	-
TNF-α	Leu 120, Ser Gly 121, Tyr 151, Gln 61	Leu 55, Val 123, Leu A 57, Leu B 57, Tyr 59, Tyr 119

essential amino acid residues, as summarized in Table 4 and Figure 7.

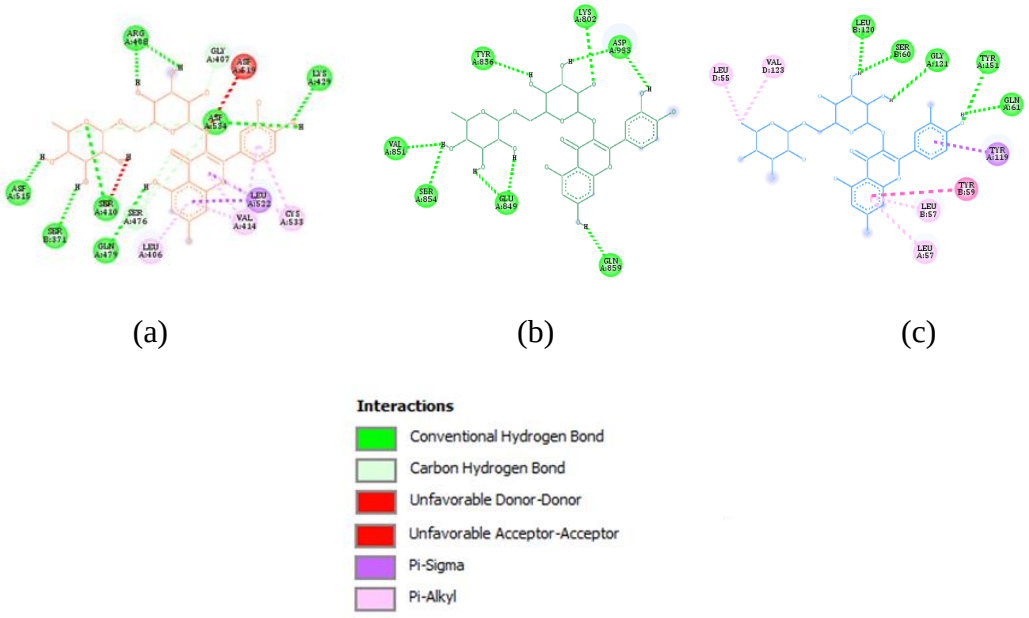
Discussion

The study used network pharmacology to systematically analyze the mechanism of action of Rutin in the treatment of Diabetic mellitus. The resulting PPI network has 17 common targets from cross-matching between genes of rutin and Diabetic mellitus. Three key targets, MTOR, PIK3R1, and NFKB1, have high network value. Thus, we speculate that the effective component of rutin may have high network value. Thus, we speculate that the effective component of Rutin may have pharmacologic activities in the treatment of Diabetic mellitus through these targets. Diabetic mellitus is a multifactorial disease characterized by increase blood sugar levels resulting inadequate insulin secretion or insulin resistance (Lee et al., 2020). Previous research has demonstrated that the pathophysiology of metabolic syndrome,



(a) (b) (c)

Figure 6. Visualization of the results of docking method validation, (a) re-docking of NF-KB native ligand, (b) re-docking of PI3K native ligand, (c) re-docking of TNF-α native ligand. Note: blue color for re-docked ligand, and green for original ligand.



(a) (b) (c)

Figure 7. Two-dimensional interaction of rutin with (NF-KB), (b) PI3K, and (c) TNF-α essential amino acid residues

obesity, and diabetes is significantly influenced by the mammalian target of rapamycin (mTOR) signaling pathway. Most human diseases, including cancer, diabetes, and genetic disorders are frequently accompanied with increased mTOR activity (Yarahmadi et al., 2022).

The mTOR is a serine/threonine protein kinase,which is highly conserved in structure and function, belonging to a phosphatidylinositol 3-kinase (PI3K)-related family member. mTOR mainly exists in the form of two complexes in vivo: mTORC1, which regulates cell proliferation and metabolic reactions and mTORC2. At present, a large number of studies have confirmed that the PI3K/Akt/mTOR signaling pathway (Granata et al., 2023). is related to some complications of diabetes (Liu et al., 2020). Physiologically, insulin and insulin-like growth factors (IGF) activate mTORC1 through the IRS/PI3K/Akt pathway.

In this network pharmacology study, we found that PIK3R1 are olso involved DM. Phosphoinositide 3-kinases (PI3Ks) are signaling molecules that play an imperative role in the regulation of metabolic homeostasis and insulin sensitivity (Tsay & Wang, 2023). PI3K is essential for signalling through the insulin and IGF-1 RTKs, making it central to the metabolic effects of insulin including glucose uptake and glycogen synthesis (Fox & Mott, 2020). Imporer functioning of PI3K/Akt insulin signaling pathway result in insulin resistance, leading to reduce function of β-cell. Enrichment analysis also revealed significant enrichment of genes in pathways such as Insulin resistance, Type II DM, and insulin signaling pathway (Prakash & Ramachandra, 2022).

Study has found that NFKB1 variant were involved in interaction with DM gene. The diverse role of the family of NFKB1A genes has been studied in type 2 diabetic associated with impairment of glucose metabolism. NFKB1A is the gene expression regulatory factor for many pro-inflamatorry proteins (Raza et al., 2022). NFKB1 are important gene that differentially express in Type 2 Diabetic Mellitus (Rahman et al., 2020).

From the results of rutin docking, Nuclear Factor Kappa-B has the most negative free binding energy compared to other receptors. Therefore, routine is considered to have more potential to bind to Nuclear Factor Kappa-B (NF-KB). The influence of NF-KB in regulating blood glucose levels is through Beta Cell Apoptosis. Pancreatic beta cells are responsible for insulin production, with apoptosis influenced by NF-KB, loss of pancreatic beta cells contributes to a decrease in insulin levels, which worsens hyperglycemia (Romeo et al., 2002).

With the potential to inhibit the NF-KB pathway, it is the same as antidiabetic drugs, such as metformin, which has an anti-inflammatory effect by inhibiting the NF-KB pathway. Metformin works by reducing oxidative stress and increasing inulin

sensitivity, which can help control blood glucose (Ibrahim et al., 2022).

On PI3K, PI3K plays a key role in the insulin signaling pathway. When insulin binds to its receptor on the cell surface, it activates PI3K, which then triggers a series of reactions that lead to the translocation of GLUT4 (glucose transporter type 4) to the cell membrane. GLUT4 is responsible for taking glucose from the blood into cells, especially in muscle and adipose tissue. If PI3K activity is impaired, GLUT4 translocation will decrease, leading to decreased glucose uptake by cells and increased blood glucose levels (Chandra et al., 2001).

When compared with antidiabetic drugs, Metformin increases insulin sensitivity through activation of AMP-activated protein kinase (AMPK), which in turn can increase PI3K activity and improve insulin signaling. Metformin also reduces glucose production in the liver and increases glucose uptake by muscle, in part through increasing PI3K pathway activity (Ibrahim et al., 2022).

And the next receptor, TNF-α causes disruption in the insulin signaling pathway. This activates stress kinases, including c-Jun N-terminal kinase (JNK) and IKK kinase, which then phosphorylate insulin receptor substrate (IRS) proteins at serine residues. This phosphorylation inhibits the IRS protein's ability to transduce insulin signals, meaning that insulin sensitivity is lower (Peraldi et al., 1996).

When compared with antidiabetic drugs such as Metformin. Metformin increases insulin sensitivity and reduces hepatic glucose production; metformin also has anti-inflammatory properties. Metformin can reduce the expression level of TNF-α, thereby reducing systemic inflammation and insulin resistance associated with it (Ibrahim et al., 2022).

Apart from that, the potential activity of Rutin against each protein is also influenced by the interactions that occur between the ligand and amino acid residues. In this study, the results show that each ligand has a different type of interaction which is also mediated by different amino acid residues. All these amino acid residues interact with the ligand through direct and indirect interactions. The interactions that occur most frequently are hydrogen, sigma, alkyl, and pi bonds. This interaction plays an important role in the stability of the bond between the ligand and the protein. Hydrogen bonds, formed between hydrogen atoms and electronegative atoms, provide significant strength and specificity to the binding, contributing 2-10 kcal/mol to the binding energy (Du et al., 2016). Pi-sigma interactions occur between aromatic rings and nearby sigma bonds, while pi-alkyl interactions involve aromatic rings and alkyl groups. These interactions, though generally weaker than hydrogen bonds, are important in determining ligand orientation and selectivity, especially in hydrophobic binding pockets (Lu et al., 2019). The combination of these

interactions works synergistically to enhance both the strength and specificity of ligand-protein complexes. Understanding these interactions is vital for drug design, as it allows researchers to optimize ligand structures for improved binding affinity and specificity, potentially leading to more effective drugs with fewer side effects (Ferreira de Freitas & Schapira, 2017). By considering these various interaction types, scientists can better predict and manipulate the behavior of drugs in biological systems, ultimately advancing the field of drug discovery and development.

CONCLUSION

The study employing network pharmacology and molecular docking approaches has shed light on the potential antidiabetic mechanism of rutin. The network pharmacology analysis identified mTOR, PIK3R1, and NFKB1R as key targets of rutin, which are involved in crucial pathways related to diabetes, including insulin signaling, insulin resistance, type 2 diabetes mellitus, B receptor signaling pathways, AGE-RAGE signaling pathway in diabetic complications, and pancreatic cancer. Molecular docking results further corroborated these findings, demonstrating stable binding of rutin to NF-κB, PI3K, and TNF-α with favorable estimated free binding energy values of -8.54 kcal/mol, -8.01 kcal/mol, and -6.22 kcal/mol, respectively. These findings suggest that rutin may exert its antidiabetic effects through multiple pathways and targets, highlighting its potential as a promising therapeutic agent for diabetes management. Further experimental validation is warranted to confirm these computational predictions and fully elucidate rutin's mechanism of action in diabetes treatment.

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