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Original Article
Livestock Diseases

Exploring the mechanism of quercetin treatment of bovine viral diarrhea mucosal disease based on network pharmacology and *in vitro* validation[1]

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ABSTRACT.- Wang S., Zhang P., Huang X., Yang H., Li W., You H., Guo W., Chen L., Wang Z., Chen X., Li L., Yang X., Liu X. & Wang H. 2024. **Exploring the mechanism of quercetin treatment of bovine viral diarrhea mucosal disease based on network pharmacology and *in vitro* validation.** *Pesquisa Veterinária Brasileira 44:e07546, 2024.* Branch of Animal Husbandry and Veterinary, Heilongjiang Academy of Agricultural Sciences, Qiqihar 161006, China. E-mail: scizyyx@126.com

Bovine viral diarrhea virus (BVDV) can cause acute and persistent infections in cattle, resulting in significant economic losses to the livestock industry each year. Targeted antiviral therapy is an effective strategy. This study was based on network pharmacology, molecular docking techniques and *in vitro* studies to investigate quercetin's mechanism in treating bovine viral diarrhea/mucosal disease (BVD-MD). The network topology analysis was carried out using Cytoscape 3.9.0 software to construct the network of "Chinese medicine ingredients-target-diseases". Protein interactions were explored and analyzed using the String system (PPI). GO and KEGG pathway analysis of the intersected targets was performed using Bioconductor software. The molecular docking and molecular dynamics simulation methods were used to reveal the degree of binding of quercetin to key target genes. Western blot and indirect immunofluorescence were used to characterize the antiviral effects of quercetin. This study utilized network pharmacological analysis, identifying 22 targets associated with BVD-MD. The results of the KEGG pathway showed that quercetin was closely related to Ras and MAPK signaling pathways of BVD-MD. Molecular docking results showed that SRC, NS5B, NOX4 and XDH were the key targets of quercetin in the treatment of BVD-MD. Through network pharmacology, molecular docking and *in vitro* experiments, quercetin was demonstrated to combat bovine viral diarrhea mucosal disease through key targets of SRC, MAPK1, GSK3B, NS5B and E2. Molecular dynamics analysis showed that quercetin exhibited complex stability with NS5B. This study provides a theoretical and experimental basis for quercetin treatment of BVD-MD and later drug development.

INDEX TERMS: BVD-MD, quercetin, web-based drug screening, molecular docking, antiviral.

INTRODUCTION

Viruses in the pestivirus genus are important economic animal pathogens, including bovine viral diarrhea virus (BVDV), border disease virus (BDV) and classical swine fever virus (CSFV) (Lindenbach et al. 2007). Bovine viral diarrhea/mucosal disease (BVD-MD) is caused by bovine viral diarrhea/mucosal disease virus (BVDV) and occurs mainly in cattle, but also in sheep, pigs, goats, and other even-toed ungulates. Pestivirus is most closely related to hepatitis C virus (HCV), a serious and persistent threat to global health (Shepard et al. 2005).

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Control and prevention of BVDV infection should be combined with systematic vaccination and culling of persistently infected cattle (Newcomer & Givens 2013). However, due to the wide antigenic diversity of the virus, immunity is complex and cannot target the emergence of persistently infected animals (Fulton et al. 2003, Newcomer et al. 2017). At the same time, no antiviral products are currently licensed for use in cattle. This suggests an urgent need for an effective preventive or therapeutic agent to combat a virus closely associated with commercial cattle production (Newman et al. 2022).

BVDV single-stranded RNA is translated by host cells into polyproteins, which are processed by viral and host proteases and sheared into four structural proteins (proteins C, Erns, E1, and E2) and eight functional proteins (Al-Kubati et al. 2021). The E2 glycoprotein binds to a cell surface receptor (CD46 or CD81), causing membrane fusion to begin and releasing BVDV single-stranded RNA in the host cytoplasm (Liang et al. 2003, Pierson & Kielian 2013). It has been reported that viral envelope proteins are important targets for developing antiviral drugs, and drug designs based on this structure have been successfully used to identify envelope protein-small molecule ligands that block the entry of flaviviruses (Zhou et al. 2008, Kampmann et al. 2009, Leal et al. 2017). In addition, non-structural viral proteins have been most suppressed in drug discovery over the past few decades, such as the RNA-dependent RNA polymerase NS5B (RdRp), the NS4a protease, and the NS3 helicase (Bollini et al. 2018). Studies have shown that antiviral drugs targeting the virus polymerase NS5B can immediately protect the virus from attack (Newcomer et al. 2012), and recent studies have also demonstrated that by targeting NS5B, BVDV virus replication can be inhibited, with good antiviral effects *in vivo* and *in vitro* (Chen et al. 2023). Therefore, targeted antiviral drugs are an option for preventing and treating BVDV outbreaks.

Quercetin is a natural flavonoid compound that can be extracted from many plants' flowers, leaves and fruits (Chen et al. 2022). Studies have shown that quercetin has potent anti-inflammatory, antioxidant, immunomodulatory and antiviral effects and can treat liver, heart, spleen, lung, kidney, orthopedic diseases, and nervous system diseases in cell and animal experiments (Bachmetov et al. 2012, Xu et al. 2019, Gangwar et al. 2021). Quercetin has shown antiviral activity against several zoonotic coronaviruses, including SARS-coronavirus and hepatitis B virus (HBV) (Rojas et al. 2016, Bachar et al. 2021). The studies demonstrated that quercetin can inhibit Hsp70 and block BVDV infection in the early stage of viral infection (Chen et al. 2022). In the preliminary study, we first searched the database for traditional Chinese medicine (TCM) components and genes of host animals associated with BVDV infection. The results showed that quercetin was the core ingredient of Chinese medicine. Therefore, quercetin is a promising candidate for developing antiviral drugs against a wide range of viruses, but the mechanism of action of quercetin in the treatment of BVDV is not known.

Network pharmacology is a new subject based on systems biology theory, which analyzes biological systems and selects specific signal nodes for multi-target drug molecular design (Liu et al. 2019, Niu et al. 2019, Pinzi & Rastelli 2019). In this study, network pharmacology was used to conduct a cluster analysis of quercetin's action targets and disease targets related to BVD-MD were conducted to obtain the intersection targets of quercetin's action on BVD-MD. Then, analysis software was used to conduct molecular docking and metabolic pathway analysis of the combination-target sites and determine the molecular-target-pathway mechanism of quercetin's treatment of BVD-MD. It provides a reference for further research and new drug development.

MATERIALS AND METHODS

Ethical approval. Since all the data were obtained from database searches, this study did not perform any animal experiments. It was not necessary to submit to the local committee on animal use.

Viruses and reagents. BVDV was from the China Microbial Strain Conservation Center (CMSC) (Bio-85318). Quercetin was purchased from Fenghe Biological Company (Shanxi, China).

Quercetin target collection. The compound's Simplified Molecular Input Line Entry System (SMILES) was obtained using the PubChem database[6]. Then, the SMILES number was imported into the structural similarity prediction target database to predict its effective target[7].

Prediction of BVD-MD targets. The keywords "bovine viral diarrhea-mucosal disease" were entered into GeneCards[8] and OMIM[9] databases for search. This action displayed the genes associated with the disease. After downloading, the Gene Symbol information related to the disease was extracted, and the initial screening proceeded. The procedure used was: Input your keywords into the OMIM database search. Begin by visiting the homepage and clicking "Gene Map" to access the screening page. In the search box, enter the name of the disease of interest. Once the targets related to the disease are gathered, combine them all and remove duplicate genes. The resulting list of combined targets represents the disease targets for this study.

Acquisition of quercetin and BVD-MD intersecting targets. Venny software[10] was used to obtain the intersection targets of active compounds and diseases, then used as potential key targets for treating diseases.

"Component-target-disease" network analysis. The compound gene "network" file was prepared, the file was typed, and Cytoscape3.9.0 software was used to import related files for network topological analysis. The target pattern, color, transparency, and size according to the degree value were adjusted, and the network diagram of "components, target sites, and diseases" was constructed.

PPI network construction and network topology analysis. The intersection gene was imported through the String[11] platform, the object was set as (*Homo sapiens*), the highest confidence was 0.900, and the free gene node was hidden to obtain the protein interaction relationship. The procedure used was: Import the result into Cytoscape 3.9.0 software and select the network analyzer to get network topology parameters. Then, import the downloaded TSV file into Cytoscape software to make a PPI chart and select the top 10 core targets according to the Degree value.

GO and KEGG enrichment analysis. The bioinformatics open-source software Bioconductor[12] was utilized to install the clusterProfiler, Stringin, and Pathview packages within the R software, which was then used to analyze the GO and KEGG functional enrichment of biological processes (BP) and visualize them.

[6] Available at <https://pubchem.ncbi.nlm.nih.gov/> Accessed on Apr. 15, 2024.
[7] Available at <http://www.swisstargetprediction.ch> Accessed on Apr. 15, 2024.
[8] Available at <https://www.genecards.org/> Accessed on Apr. 15, 2024.
[9] Available at <https://www.omim.org/> Accessed on Aug. 29, 2024.
[10] Available at <https://bioinfogp.cnb.csic.es/tools/venny/> Accessed on Aug. 29, 2024.
[11] Available at <https://string-db.org/> Accessed on Aug. 29, 2024.
[12] Available at <http://www.bioconductor.org/> Accessed on Aug. 29, 2024.

Molecular docking. The large and small molecules that rank high in topological analysis are molecularly docked. Protein crystal structures are obtained from the RCSB PDB or Uniprot. ATP1A1 (PDB ID: 4xe5); E2 (PDB ID: 4ILD); NS5B (PDB ID: 1S48); XDH (PDB ID: 1FO4); AKR1B1 (Uniprot ID: P16116); FLT3 (Uniprot ID: F1MRU0); GSK3B (Uniprot ID: A0A3Q1NDU7); IGF1R (Uniprot ID: Q05688); NOX4 (Uniprot ID: F1MQX5); SRC (Uniprot ID: E1BIM8). Protein crystal structures were dewatered, and hydrogenation and receptor structures were prepared using AutodockTools1.5.6. Visualization was performed using PyMol 2.4 software.

Molecular dynamics simulation. Protein-ligand complexes were simulated using Gromacs 2022.3 molecular dynamics simulation software. We initially utilized the steepest descent method during the simulation to minimize the system's energy, eliminating possible conformational stresses and geometric irregularities. Subsequently, we conducted canonical ensemble and constant-pressure, constant-temperature equilibrations, each consisting of 100,000 steps. We maintained a coupling constant of 0.1 ps and a duration of 100 ps to ensure the system's stability regarding temperature and pressure. Finally, a free molecular dynamics simulation was performed, comprising 50,000,000 steps with a step size of 2 fs, spanning 100 nanoseconds, to capture the system's dynamic behavior fully. The respective trajectories of complexes were analyzed from root-mean-square deviation (RMSD), root-mean-square fluctuation (RMSF), radius of gyration (Rg), and hydrogen bonds (HBs).

Effect of quercetin on the replication of BVDV virus. Based on recent research findings, we treated MDBK cells with varying concentrations of quercetin (100 and 200μmol/L) for 6 h. Afterward, the BVDV-CP stock solution (MOI=1) was introduced, incubated for 1 h for adsorption, and supplemented with DMEM culture solution. After 48 h, RNA was extracted and analyzed by Western blotting (Chen et al. 2022).

MDBK cells were cultured, and when their density reached approximately 25%, they were treated with either quercetin (100 and 200μmol/L) or infected with the BVDV-CP virus. Next, the cell culture medium was discarded, and the cells were washed with PBS. Cells were fixed with 4% paraformaldehyde for 30 min, followed by three PBS rinses. Afterward, the cells were permeabilized with 0.5% triton X-100 at room temperature for 15 min, washed with PBS, and blocked with 5% bovine serum albumin (BSA) at 37°C for 1 h. Subsequently, at 37°C, the cells were incubated with antibodies that resist viral proteins for 1 h and rinsed with PBS thrice. The treated cells were further incubated with a secondary antibody labeled with Alexa Fluor 594 for 1 h. Finally, the cells were stained with DAPI at room temperature for 10 min, enabling the observation of red viral proteins and blue nuclei using confocal laser scanning microscopy.

Statistical analysis. Statistical differences were determined by Student's t-test using GraphPad Prism 9.0 software. For all experiments, differences were considered to be statistically significant at the levels of *p<0.05, indicating a significant difference between the two sets of data; **p<0.01, indicating a very significant difference between the two groups of data; and ***p<0.001, indicating an extremely significant difference between the two sets of data.

RESULTS

Construction and comparative analysis of potential target data of quercetin and BVD-MD

The quercetin compound was sourced from the PubChem database, leading to the screening of potential quercetin targets upon import into the database. Simultaneously, BVD-MD-related target genes were screened through the OMIM and GeneCards databases, acquiring relevant targets after eliminating duplicate genes. A Venn diagram was employed to analyze the shared targets between quercetin and BVD-MD-related pathogenesis, ultimately identifying 22 targets common to quercetin and BVD-MD-related pathogenesis (Fig.1).

Results of network analysis of components, targets and diseases

Using Cytoscape 3.9.0 software, network topology analysis was performed to construct the "component-target-disease" network map according to the degree value. This network makes observing relationships between ingredients, targets, and diseases easy. These results suggest that quercetin's pharmacological effect in treating BVD-MD results from multi-component and multi-target action (Fig.2).

PPI network construction and network topology analysis results

The protein interaction was obtained by introducing the intersection gene through String. The network topology parameters were obtained using Cytoscape 3.9.0 software.

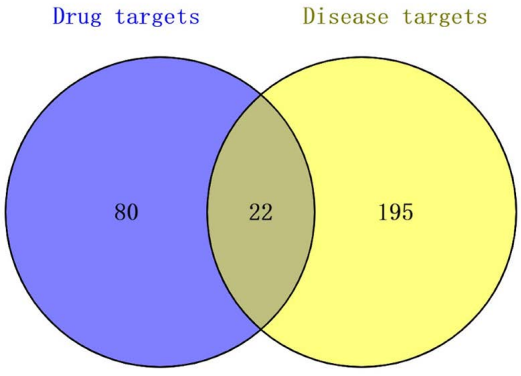

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Fig.1. Quercetin target and bovine viral diarrhea/mucosal disease (BVD-MD) related pathogenesis target Wayne diagram.

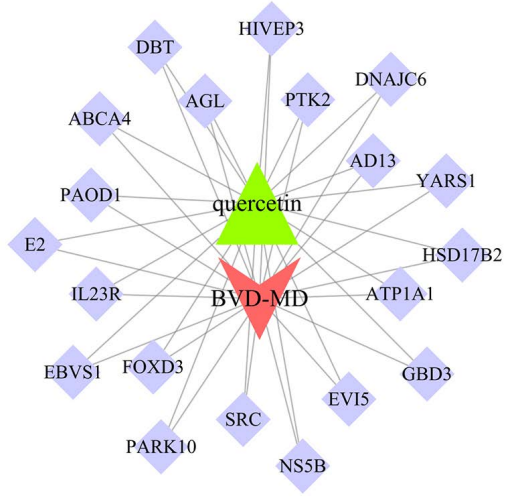

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Fig.2. Component, target, and disease network map. Quercetin (triangular node), disease (arrow node), target (quadrilateral node). The connections represent the interaction between the three.

Core targets were filtered according to degree value. Finally, 12 key quercetin targets on BVD-MD were acquired, including SRC, GSK3B, IGF1R, NOK4, NS5B, and E2 (Fig.3-5).

GO and KEGG pathway enrichment analyses

GO and KEGG functional enrichment analysis of biological processes was performed to understand further the functions of selected core targets and their roles in signaling pathways. GO functional enrichment analysis yielded 743 GO entries (p<0.05), of which 595 were bioprocesses (BP). The major biological processes enriched were positive regulation of endothelial cell chemotaxis, regulation of endothelial cell chemotaxis, phospholipid translocation, lipid translocation, regulation of membrane lipid distribution, cell communication involved in cardiac conduction, export across the plasma membrane, positive chemotaxis, and regulation of cardiac conduction. The cellular composition (CC) entries were 55, and the major cellular components were dihydrolipoyl dehydrogenase complex, sodium:potassium-exchanging ATPase complex, tricarboxylic acid cycle enzyme complex, cation-transporting ATPase complex basolateral plasma membrane, photoreceptor disc membrane, ATPase dependent transmembrane transport complex, membrane raft, membrane microdomain, and postsynaptic density. Entries for molecular function (MF) were 93. The major molecular functions were ATPase-coupled intramembrane lipid transporter activity, ATPase-coupled transmembrane transporter activity, primary active transmembrane transporter activity, intramembrane lipid transporter activity, phospholipid transporter activity, transmembrane receptor protein tyrosine kinase activity, transmembrane receptor protein kinase activity, active transmembrane transporter activity, lipid transporter activity, and protein tyrosine kinase activity. The KEGG pathway enrichment screen yielded 52 signaling pathways (p<0.05) involving the Ras, MAPK, and PI3K-Akt signaling pathways, among others (Fig.6-7).

Molecular docking result

The macromolecules ranked in topological analysis, and small molecules (quercetin) were submitted to molecular docking. It is generally believed that the more stable the conformation of ligands and receptors, the lower the energy and the greater the possibility of interaction. The results of molecular docking showed that quercetin had good binding

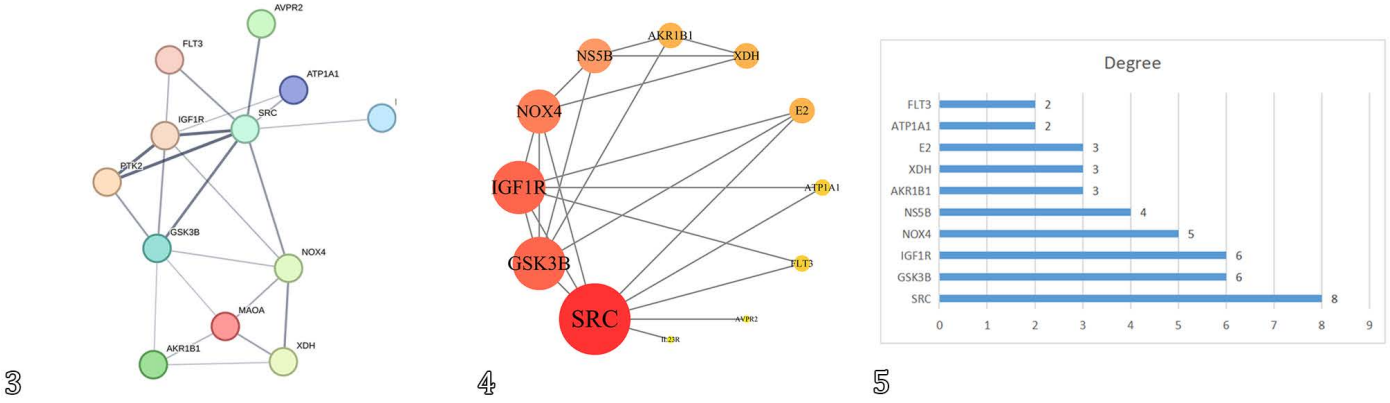

Fig.3-5. Protein interaction network topology screening (PPI). (**3**) PPI original drawing. (**4**) PPI. (**5**) Core target. The size and color of the node are related and adjusted according to the degree value. The darker the color, the larger the node, and the greater the degree value.

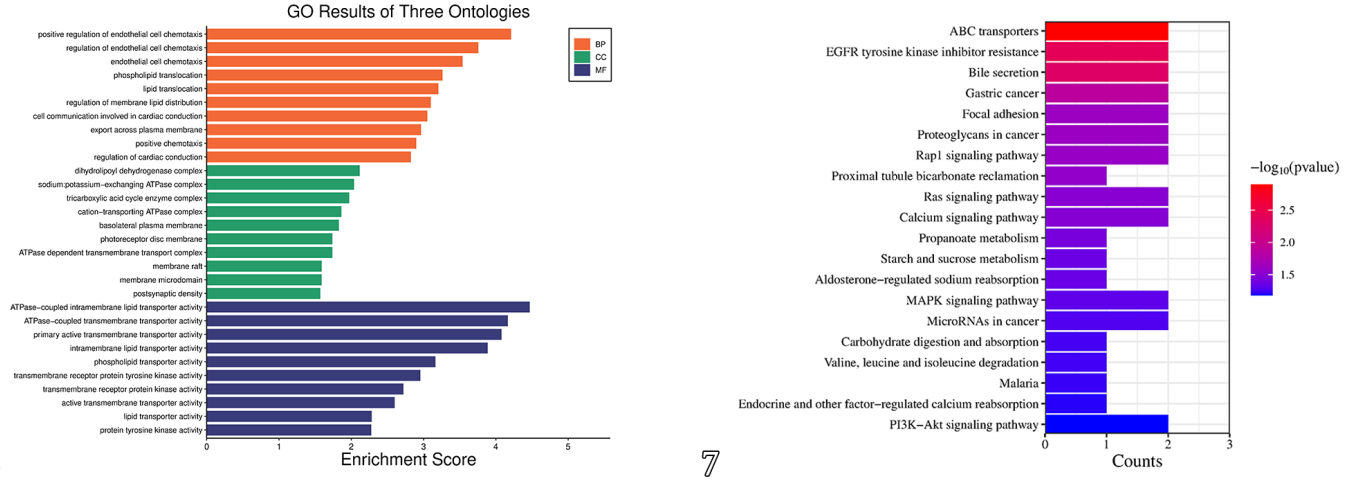

Fig.6-7. GO and KEGG pathway enrichment analysis. (**6**) GO results of three ontologies. (**7**) KEGG pathway enrichment analysis. BP = biological process, CC = cellular component, MF = molecular function.

activity with core protein macromolecular receptors (SRC, NS5B, NOX4, XDH) (Fig.8-17). The docking results in Table 1 provide details on binding energy, interaction forces, and bond lengths for molecular docking.

Molecular dynamics simulation results

Based on the analysis of molecular docking results, the protein receptor NS5B bound to the small molecule quercetin was chosen for MD simulation lasting 100 ns. The RMSD

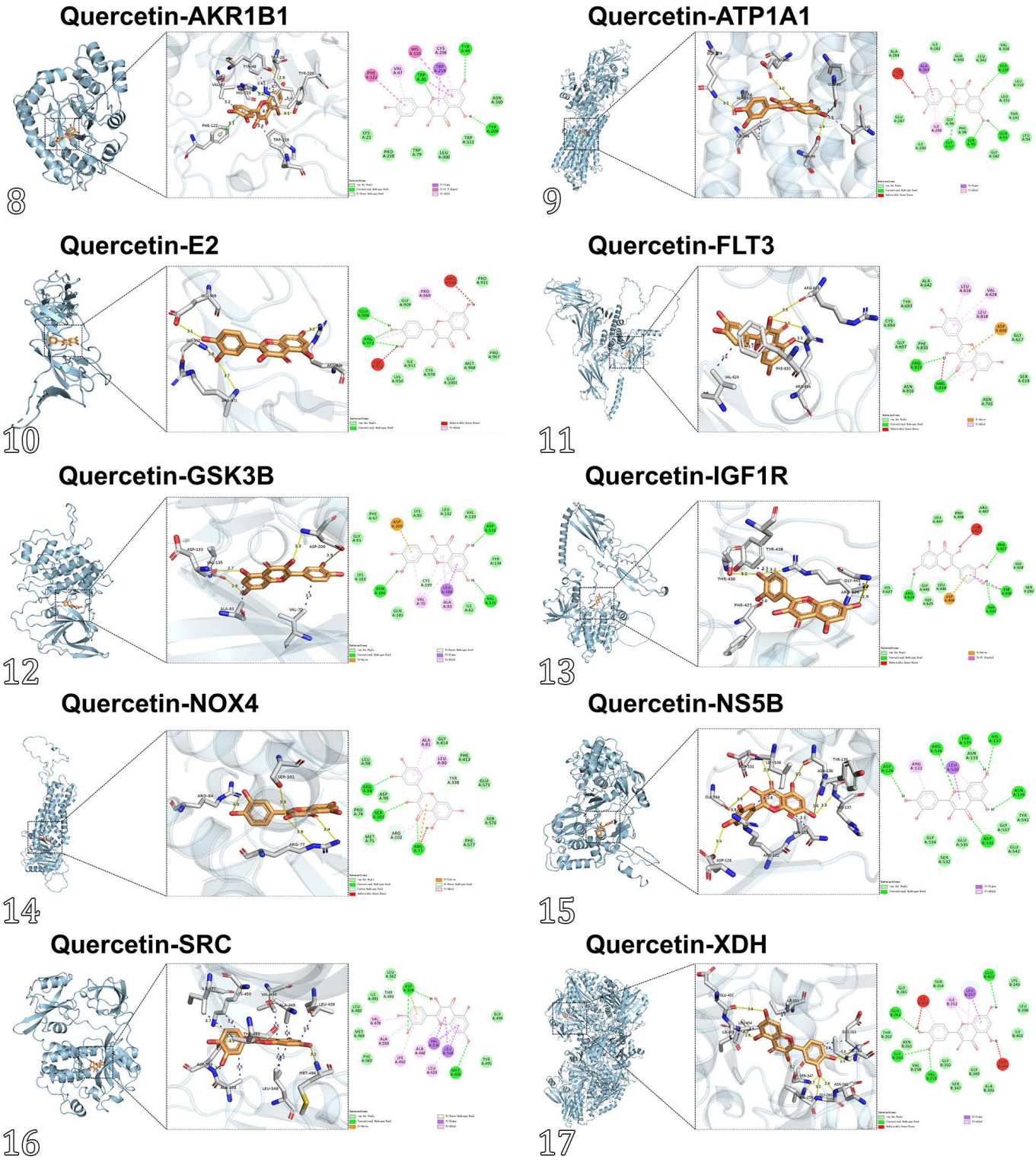

Fig.8-17. Verification of docking between small molecules and key targets. (**8**) Quercetin-AKR1B1. (**9**) Quercetin-ATP1A1. (**10**) Quercetin-E2. (**11**) Quercetin-FLT3. (**12**) Quercetin-GSK3B. (**13**) Quercetin-IGF1R. (**14**) Quercetin-NOX4. (**15**) Quercetin-NS5B. (**16**) Quercetin-SRC. (**17**) Quercetin-XDH. The receptor was chosen as the target protein, with quercetin selected as the ligand. Hydrogen bonds (yellow), hydrophobic interactions (blue), and π-cation/stacking (green) interactions are depicted.

curve was utilized to monitor the dynamic structural changes of the complex over time, enabling precise assessment of its stability. Figure 18 illustrates that the curve stabilizes between 20-100 ns, signifying the consistent stability of the small molecule-protein receptor complex during this period. This result provides valuable reference information for subsequent biochemical studies and drug design. It helps us better understand the interaction mechanisms between these molecules and how these interactions can be utilized to design more potent and stable biological drugs. The experimental data for root mean square fluctuation (RMSF), radius of gyration (Rg), and hydrogen bonds (HBs) are provided in the supplementary material.

***In vitro* validation of quercetin inhibition of viral replication**

Quercetin's ability to inhibit BVDV virus replication was assessed by viral proteins E2 using specific antibodies. The findings indicated that quercetin effectively hindered BVDV virus replication. To further validate these results, indirect immunofluorescence detection was conducted. The results demonstrated that 100μmol/L and 200μmol/L quercetin reduced the viral expression, with 200μmol/L quercetin producing the most pronounced effect (Fig.19-20 and 21).

DISCUSSION

BVD-MD caused by BVDV affects cattle population health and breeding in many countries around the world, causing huge economic losses (Newcomer & Givens 2016, Pinior et al. 2017, Richter et al. 2017). Because BVD-MD can cause miscarriage, infertility or embryo death, it significantly reduces reproductive performance and increases premature elimination (Newcomer et al. 2021). Therefore, BVD-MD is also considered, at present, one of the most expensive cattle diseases (Newcomer et al. 2021, Newman et al. 2022). While vaccines are an essential tool for controlling many bovine viral pathogens, despite being in use for decades, in some cases, they have proven inadequate to control related diseases (Newman et al. 2022). The continued development of targeted antiviral drugs is another tool to prevent losses associated with BVDV infection (Peng et al. 2022). Plant-derived compounds and plant extracts have high biocompatibility and safety and have great application potential. Previous studies have preliminarily demonstrated that plant compound quercetin can inhibit BVDV virus replication, but its mechanism of action on BVDV remains unclear (Chen et al. 2022). This study employed network pharmacology and molecular docking techniques to

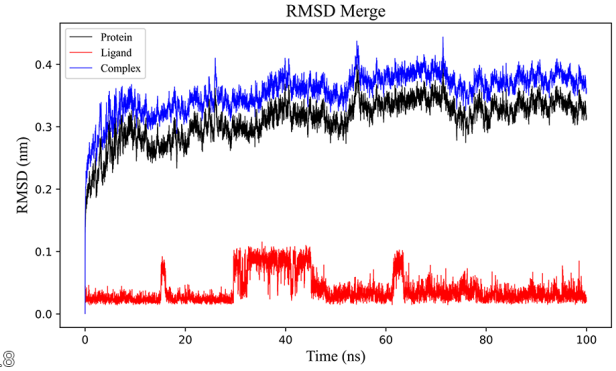

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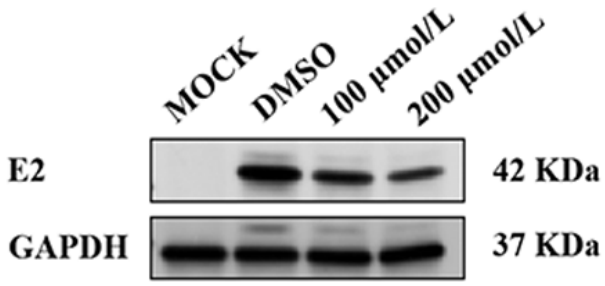
Fig.18. Root-mean-square deviation (RMSD) curves of small molecule-protein receptor docking complexes.

Table 1. Interaction of quercetin with macromolecules

Macromolecule	Binding energy (Kcal/mol)	Hydrogen bonds	Hydrophobic interaction	π-Stacking
AKR1B1	-7.9	Tyr 48A (2.81 Å), Tyr 209A (3.09 Å)	Trp 20A (3.80 Å, 3.68 Å), Val 47A (3.23 Å), Tyr 48A (3.55 Å), Tyr 209A (3.43 Å), Trp 219A (3.42 Å)	His 110A (5.25 Å), Phe 122A (5.12 Å)
ATP1A1	-8.0	Gln 93A (2.71 Å), Ser 99A (2.88 Å), Gln 279A (3.16 Å), Glu 360A (4.01 Å)	Thr 141A (3.91 Å), Ala 283A (3.63 Å), Ile 286A (3.65 Å)	
E2	-6.9	Glu 908A (3.10 Å), Arg 936A (3.22 Å), Gly 952A (2.93 Å), Arg 971A (3.68 Å)		
FLT3	-7.6	Arg 815A (3.51 Å), Arg 834A (3.67 Å, 3.28 Å)	Val 624A (3.60 Å), Phe 830A (3.68 Å)	
GSK3B	-8.4	Asp 133A (2.74 Å), Asp 200A (3.29 Å, 3.87 Å), Val 135A (2.90 Å)	Val 70A (3.51 Å), Ala 83A (3.62 Å)	
IGF1R	-7.6	Phe 427A (3.01 Å), Arg 626A (3.16 Å, 2.95 Å), Thr 430A (3.20 Å), Gly 445A (4.03 Å)	Tyr 438A (3.26 Å)	
NOX4	-8.8	Arg 77A (3.37 Å, 3.89 Å), Arg 84A (3.98 Å), Ser 101A (3.51 Å)		
NS5B	-8.9	Asp 126A (3.42 Å), Arg 132A (3.83 Å), Tyr 135A (3.62 Å), Asn 136A (3.21 Å), His 137A (3.35 Å), Asp 531A (2.95 Å), Gly 534A (3.92 Å)	Asn 133A (3.34 Å), Leu 530A (3.80 Å)	
SRC	-8.6	Lys 450A (3.99 Å), Met 496A (3.18 Å), Asp 559A (2.96 Å, 3.17 Å)	Leu 428A (3.62 Å), Val 436A (3.50 Å), Ala 448A (3.55 Å), Lys 450A (3.39 Å), Ile 491A (3.72 Å), Thr 493A (3.57 Å), Leu 548A (3.75 Å, 3.22 Å), Ala 558A (3.50 Å)	
XDH	-9.7	Glu 263B (2.99 Å), Val 259B (2.72 Å), Gly 260B (3.15 Å), Asn 261B (2.80 Å), Ser 347B (3.20 Å), Glu 402B (3.79 Å), Leu 404B (2.79 Å), Ile 403B (3.95 Å), Ile 264B (2.95 Å)	Glu 263B (3.70 Å), Ile 264B (3.94 Å), Ile 353B (3.78 Å), Ile 403B (3.95 Å)	

investigate how quercetin acts on BVD-MD. This study aimed to identify the targets it affects and the associated metabolic pathways. Ultimately, the goal was to unveil the molecular mechanism by which quercetin treats BVD-MD.

The protein encoded by the SRC gene belongs to the SRC family of kinases (SFKs), consisting of nine members: SRC, LYN, FYN, LCK, HCK, FGR, BLK, YRK and YES. SRC is currently the most studied member and the protein most closely associated with human diseases. SRC proteins are non-receptor tyrosine kinases that multiple signal transduction pathways can activate. After activation, SRC kinases are activated by phosphorylation of tyrosine residues of corresponding target proteins, thereby activating the complementary signal pathways, including MAPK, STAT, PI3K/AKT and EGFR. The abnormal activation



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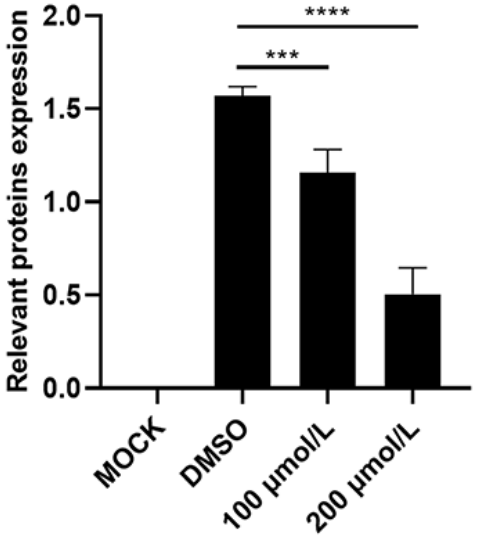

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Fig.19-20. Quercetin inhibits bovine viral diarrhea virus (BVDV) protein expression. (**19**) Expression level of BVDV-E2 protein. (**20**) Analysis of protein gray value.

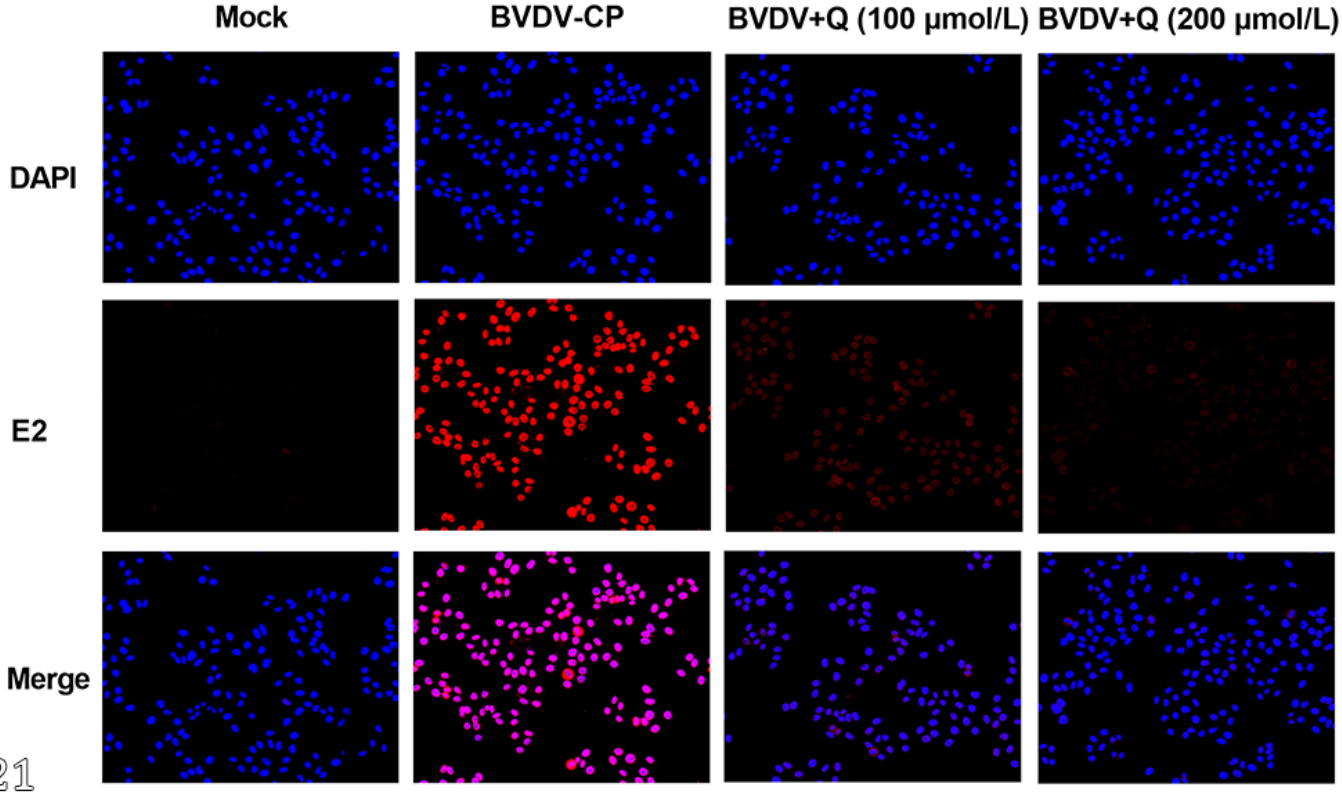

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Fig.21. Verification of the inhibitory effect of quercetin on bovine viral diarrhea virus (BVDV) replication.

of the SRC protein is linked to numerous tumors, and its activity level closely correlates with tumor development by promoting cancer cell proliferation, restructuring cancer cell protein frameworks, initiating invasion and metastasis mechanisms, and inducing angiogenesis (Peng et al. 2022). It was found that sarcosine ethanol extract and its active ingredient, quercetin, inhibited LPS-induced macrophage activation by targeting Syk/Src/IRAK-1 (Yang et al. 2014). In this study, SRC is identified as a critical core target.

Studies have found that flavonoids (quercetin, luteolin and kaempferol) are the main active components of *Ginkgo biloba* leaf extract (EGb). The candidate targets GSK3β and MAPK14, as the primary therapeutic targets of EGb, play an anti-aging role by inhibiting oxidative stress, inhibiting inflammation and improving insulin resistance (Liu et al. 2020). In addition, it was demonstrated that GSK-3 is essential for the phosphorylation of SARS-CoV-2 nuclear capsid protein and that GSK-3 inhibition can block SARS-CoV-2 infection in human lung epithelial cells (Liu et al. 2021). Quercetin inhibited the activation of IGF1R and its downstream kinases Ekt and Erk1/2 in a dose-dependent manner in human MDA-MB-231 breast cancer cells (TNBC cell line) (Chen et al. 2021). In addition, quercetin treatment can reduce the expression of NOX (NOX1 and NOX4) in the Achilles tendon of diabetic rats, thus playing the role of antioxidant and anti-inflammatory (Yoshikawa et al. 2022). This study identified GSK3B, IGF1R, and NOX4 as significant targets of BVDV, suggesting their potential as candidate targets for future screening of anti-BVDV drugs.

RNA-dependent RNA polymerase (RdRp) is critical in viral RNA synthesis and is one of the most crucial antiviral drug targets. The non-structural protein NS5B of BVDV has RdRp activity and is involved in constructing viral replicons (Duan et al. 2020, Chen et al. 2023). Antiviral drugs that target the pestivirus virus polymerase NS5B provide immediate protection to the virus, shielding it from attacks (Newcomer et al. 2012). The previous study found that traditional Chinese medicines targeting BVDV-NS5B were screened through molecular docking and then proved that the screened Chinese medicines could effectively inhibit BVDV virus replication through *in vivo* and *in vitro* studies (Chen et al. 2023). This study found that BVDV-NS5B has good binding with quercetin through molecular docking. The stability of the complex was further evaluated using molecular dynamics simulations, revealing quercetin and NS5B to exhibit superior complex stability. Therefore, RNA polymerase NS5B emerges as a crucial target for treating BVDV.

Mitogen-activated protein kinase (MAPK) is a family of very conserved silk/threonine protein kinases in eukaryotic cells that play a role in many cellular activities, such as growth and proliferation, cell differentiation, cell motility, or cell death. The MAPK pathway has four main branches: ERK, JNK, p38/MAPK, and ERK5. JNK and p38 have similar functions related to inflammation, apoptosis and growth. ERK primarily regulates cell growth and differentiation, and its upstream signal is the widely recognized Ras/Raf protein. The three kinases used in the branching route are different and can be used as biomarkers in the pathway. Ras, Raf, MEK, and ERK proteins are critical factors in this pathway; abnormal functioning of any of these proteins can lead to severe disease. In this study, GO analysis results showed that quercetin was mainly involved in the BP of the body in treating BVD-MD. The results of the KEGG analysis showed that quercetin acted on BVD-MD mainly through MAPK and Ras signal pathways. Previous findings demonstrated that quercetin can hinder BVDV virus replication by suppressing the ERK pathway. This inhibition is closely linked to quercetin's ability to reduce oxidative stress and modulate HSP70. It can be seen that quercetin can treat BVD-MD by acting on multiple pathways.

Therefore, quercetin anti-BVDV demonstrates not only direct antiviral effects but also anti-inflammatory, immunomodulatory, and oxidative stress amelioration properties via essential pathways and core targets. This establishes a basis for targeted Chinese medicinal formulations against BVD-MD and offers reference data for future antiviral Chinese medicinal preparations within the Flaviviridae family. The limitation of this study is that the predicted results were mainly validated by network computer technology and cellular experiments, and further studies involving animal and clinical trials are still lacking. Further exploration is needed to understand better the mechanism of quercetin in treating BVDV, thereby strengthening the persuasiveness of our findings.

CONCLUSION

In this study, the mechanism of quercetin in treating bovine viral diarrhea/mucosal disease (BVD-MD) was initially analyzed using network pharmacology, molecular docking techniques, and molecular dynamics simulations. On this basis, the targeted anti-BVDV replication of quercetin was further verified *in vitro*. The methods employed above have provided evidence of the reliability of network computer technology. Furthermore, they have enhanced our understanding of quercetin's mechanism in treating BVD-MD, establishing a foundation for subsequent research. To sum up, future research on antiviral drugs can be verified by network computer technology combined with internal and external studies.

Supplementary material. Supplementary material to this article can be found online at <https://book.yunzhan365.com/sjdgu/qdix/mobile/index.html>

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