# Network Pharmacology-based Analysis on Treatment of Diabetic Retinopathy by Berberine

**Ting-Hua Wang** ( wangtinghua@vip.163.com )
Jinzhou Medical University
**Na Li**
Jinzhou Medical University
**Cong Fu**
Jinzhou Medical University
**Jin-Lin Chen**
Jinzhou Medical University
**Xi-Liang Guo**
Jinzhou Medical University

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## Abstract

The diabetic retinal injury model was established. After treatment with berberine, OCT and water maze were measured, and 10 genes were screened out by 4D-DIA quantitative proteomic analysis. Heat map difference analysis and molecular docking were performed. The key words "diabetic retinal injury" were input in PubMed, and the related genes queried were combined with the results of 10 screened proteome analysis to conduct PPI protein interaction. Then GO and KEGG analyses were performed with the interacting genes. Results :OCT results showed that the outer nucleus, inner layer and outer accessory layer of RPE were thickened after berberine treatment. The results of water maze experiment showed that after berberine treatment, the learning and cognitive function of BBR group rats was significantly higher than that of DM group on day 5. By screening proteome analysis data and making Venny cross map, 10 genes could be obtained and heat map could be made. Dennd1a and Utp6 were down-regulated in diabetes group. Atp7a, Ppl, Ogn, Nsmcel, Mme, Lmo4, Ca1 and Fn1 were upregulation, and the berberine treatment group was opposite to the diabetic group. Molecular docking results showed that berberine and berberine could form stable molecular binding patterns except Fnl, Ogn and Utp6 target proteins. 343 genes were searched through PubMed keywords, and 353 genes were combined for PPI protein interaction, and 331 genes were obtained for mutual relationship, which were analyzed by GO and KEGG. Conclusion: Berberine has protective effect on diabetic retina injury.

## Introduction

Diabetic retinopathy (DR) is caused by chronic hyperglycemia (Youngblood, Robinson et al. 2019). The number of patients with DR is increasing as the elderly population increases (Bai, Yang et al. 2021). DR is characterized by retinal ischemia, leakage of retinal vascular system, retinal inflammation, neovascularization and angiogenesis, etc., which can be seen in the changes of micro vessels (Zou, Zhang et al. 2020, Yu, Zhang et al. 2021). DR is also a common and devastating complication in patients with diabetes mellitus (DM) that can lead to microvascular injury (Chakravarthy and Devanathan 2018). Patients with DM have a 35% chance of developing DR, a devastating complication from which there is no effective and definitive treatment option to recovery to date (Wang and Zhang 2021). It is also the number one cause of blindness when the disease is severe (Horton and Barrett 2021). DR is a specific manifestation of severe microvascular injury and can induce some other disease factors (Skol, Jung et al. 2020). Studies have shown that DM initially affects the retinal neurons of patients, leading to neurodegeneration that occurs early as DR, after which patients develop more pronounced vascular abnormalities (Kim, Kim et al. 2017). DR not only brings great distress to the life of patients, but also imposes heavy economic burden to their families and influences the development of society (Kim, Kim et al. 2018). Therefore, there is an urgent need to explore an effective method to alleviate and treat DR in patients with DM.

Berberine is a bioactive alkaloid with high medicinal value isolated from a variety of Chinese herbal medicine, which is quaternary ammonium salt from proto-berberine group of isoquinoline alkaloid (Ortiz, Lombardi et al. 2014, Cicero and Baggioni 2016, Wang, Feng et al. 2017). Berberine has a variety of pharmacological effects, including antibacterial, anti-cancer activity, antiviral activity, anti-diabetes (Warowicka, Nawrot et al. 2020). More studies have shown that berberine has a wide range of bioactive plant chemicals, and berberine is involved in extensive metabolism through oral administration, resulting in very low plasma exposure to berberine (Imenshahidi and Hosseinzadeh 2019, Song, Hao et al. 2020). Furthermore, berberine not only improve the efficacy but also the safety of chemotherapy. It have also shown that berberine has good efficacy in many chronic diseases, such as cardiovascular and cerebrovascular diseases and chronic hypertension (Hou, He et al. 2020, Suadoni and Atherton 2021). Berberine has many medicinal values that deserve further study and it also plays a positive contribution to the treatment of metabolic diseases (Kumar, Ekavali et al. 2015).

The purpose of this study is to investigate the protective mechanism of berberine on the treatment of DR by animal experiments, network pharmacology, bioinformatics, and molecular docking.

## Materials And Methods

### Establishment of DR rat model and drug therapy

A total of 30 Sprague-Dawley (SD) Male rats (weighing 180-200g) were provided from the Department of Experimental Animal Science, Kunming Medical University (No. SCXK (Yunnan) K2020-0004), and were randomly divided into Sham group (n=10), DM group (n=10), and BBR group (rats with DM were treated with berberine, n=10). All procedures were performed in accordance with the guidelines and approval of the Ethics Committee of the Kunming Medical University. The animal Ethics code is KMMU20220852,The rats in DM and SAL groups were intraperitoneally injected with levomectin to establish the DM rats model. After successful modeling, the rats in BBR group were given orally with berberine (100 mg/kg/day) for 4 weeks continuously, and the rats in Sham group and DM group were given normal saline (100 mg/kg/day).

### Optical coherence tomography (OCT)

OCT is a technique that provides cross-sectional images of biological structures based on differences in the optical properties of tissues.(Katkar, Tadinada et al. 2018) Full name: SPECTRALIS OCT, Heidelberg, Germany In this experiment, OCT measurement was performed on rats in the control group, DM group and BBR group. Rats were anesthetized by intraperitoneal injection of 10 g/L pentobarbital sodium (0.6ml/100g) the pupils were dilated with compound topicamide eye drops and the corneas were kept moist with drops of hydroxy glucan eye drops. The manufacturer of compound Topicamide eye drops is Shentian Pharmaceutical (China) Co., LTD Hydroxy Glucan Eye Drops, Alcon Research LLC,Then placed in front of a corneal contact lens, the angle of light was then adjusted for fundus photography and OCT examination. All images were acquired at one optic disc interval, and the retinal thickness was measured by ImageJ software.

### Morris water maze

To detect the learning and cognitive function of rats, we conducted the Morris water maze experiment, and was performed at 1 day (d), 3 d, and 5 d after modeling (Bromley-Brits, Deng et al. 2011). A 10-cm diameter platform was placed in a black circular pool filled with water with a width of 120 cm and a height of 56 cm. An automatic camera system connected to a computer was set up above the pool and the pool was divided into four quadrants on the computer. The rats were then placed in the water and the number of times the rats crossed the platform, the time to climb the platform and the total distance were recorded using the SMART 3.0 tracking system (Harvard Bioscience (Shanghai) Co., Ltd.).

### Proteomics analysis

After 4 weeks of berberine treatment, the rats were anesthetized by intraperitoneal injection of 3% sodium pentobarbital,and the tissues of cortex, hippocampus, lung and retina were collected and stored at -80°C for proteomics analysis. The proteomics analysis of samples were performed by Shanghai Luming Biotechnology Co., Ltd. The four-dimensional data-independent acquisition (4D-DIA) labeled quantitative proteomic analysis was conducted. Total proteins in the sample were extracted and part of samples was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the other part was taken for trypsin enzymolysis. After desalting the enzymolysis peptide, the samples were identified by liquid chromatography tandem mass spectrometry (LC-MS/MS). A protein spectrum database was firstly established by the traditional DDA method, and then the mass spectrometry data of each sample was collected using DIA technique. Finally, based on the DDA database, spectrum matching, quantitative information extraction and subsequent statistical analysis were carried out.

### Venn diagram and heat map

The genes of berberine and DR were obtained from PubMed by searching the keywords ‘salidroside’ and ‘diabetic retinopathy’, and the shared genes between them were performed using Venny2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html). The heat map of protein expression in the samples were plotted by http://www.bioinformatics.com.cn, a free online platform for data analysis and visualization.

### Molecular docking

Molecular docking was performed for berberine and genes. Firstly, the protein crystal structure of the gene was searched in Protein Data Bank (PDB) (https://www.rcsb.org), and the 2D structure of berberine was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The target protein structure was dehydrated with PyMOL software to separate the ligand and receptor. With the target protein as the receptor and the active substance as the ligand, the active site of molecular docking was determined according to the coordinate of the ligand in the target protein complex. Molecular docking is performed using AutoDock Vina.

### Protein-protein interaction (PPI) network analysis

The genes were searched by entering the keyword ‘diabetic retinopathy’ on PubMed (https://pubmed.ncbi.nlm.nih.gov), and the related genes were recorded. The genes related to DR and screened by Venn diagram were imported into the STRING database (https://string-db.org/) to obtain the relevant PPI network. The obtained table was sorted out, and the duplicated genes in Node1 and Node2 were deleted, and finally genes with interactions were obtained.

### Biological function enrichment analysis

The genes screened from PPI network analysis were analyzed by Gene Ontology (GO) (http://www.geneontology.org) and Kyoto Encyclopedia of Genes and Genomics (KEGG) (https://www.kegg.jp/kegg/pathway.html) analyses using R 4.1.0 (http://www.r-project.org). It was required to pre-install activePerl.exe and R 4.1.0, and then the software package in the script was used for installation. After the installation, The genes were pasted into Symbol ID, the script was opened, the script was pasted into R software, the ID was converted, the ID was pasted into GO and KEGG analyses, and the script for GO and KEGG analyses was pasted into R 4.1.0. The biological process (BP), cellular component (CC), and molecular function (MF) involved in GO enrichment analysis and KEGG signaling pathways were obtained, and were also plotted by http://www.bioinformatics.com.cn.

### Statistical analysis

All data were entered into SPSS (version 21.0, SPSS Inc., USA) for statistical analysis. The one-way ANOVA was used for examination of the quantitative data, and the results were expressed as mean ± standard deviation (SD). $^{*}P<0.05$ was considered as statistically significant.

## Results

### Morphological changes of fundus

As shown, there was no morphological change in the control group, and the thickness of retinal pigment epithelial (RPE) outer nucleus and intima boundary-core layer in the DM group was significantly thinner than in the control group. It was shown that the thickness of RPE outer nucleus, intima boundary-core layer and external morphology layer was thickened in BBR group, compared with control and DM groups. **(Figure 1)**

### Results of Morris water maze

In order to detect the rats' learning and cognitive function, we,3,5 1 day after surgery the sham group, DM group, BBR water maze platform incubation experiment in rats, using the single factor analysis of variance and repeated measurement variance analysis in SPSS software to carry on the analysis, the results of the analysis concluded that the diabetes in rats after building, compared with the sham group, The learning and cognitive functions of rats in the

DM group were significant, P=0.002. After berberine treatment, on day 5, as shown in Figure 2, the learning and cognitive functions of rats in the BBR group were significantly higher than those in the DM group. As shown in Figure 2, according to the results of repeated measurement anOVA, the sphericity test, P=0.138, P > 0.05, satisfies the sphericity hypothesis. The sphericity test is subject to the sphericity test. The intra-subjective effect test results show are P < 0.05, indicating that there is a certain difference between different treatment groups.

**Table 1**

**Repeat measure ANOVA table**

| Repeat measure ANOVA table | | | | | |
|---|---|---|---|---|---|
| Variables | DF | SS | MF | F | P |
| Intervene | 2 | 40345.701 | 20172.850 | 168.293 | 0.01 |
| Intergroup error | 20 | 2397.346 | 119.867 | 13.077 | 0.01 |
| Time | 2 | 2268.910 | 1134.455 | 4.771 | 0.003 |
| Time*Intervene | 4 | 1655.623 | 413.906 | | |
| Repeated measurement | 40 | 3469.999 | 86.750 | | |

**Shared genes of berberine and DR**

The sequencing data were collated and the screening data were identified by protein profiling. By setting P < 0.05 and logFC absolute value > 0.5 as screening conditions, 73 proteins from the Sham group and 140 proteins from the DR-Berberine group were analyzed. There were 10 shared genes were obtained from the intersection of 73 genes in the Sham group and 140 proteins in DR-Berberine group, including Dennd1a, Utp6, Fn1, Nsmce1, Lmo4, Ca1, Ogn, Ppl, Mme and Atp7a **(Figure 3, Table 2)**.

There were 10 shared genes were obtained from the intersection of 73 genes in the Sham group and 140 proteins in DR-Berberine group, including Dennd1a, Utp6, Fn1, Nsmce1, Lmo4, Ca1, Ogn, Ppl, Mme and Atp7a

**Table 2**

**The raw data of proteomics analysis.**

| Omics raw data | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Accession** | **ProteinDescriptions** | **Gene Name** | **MW [KDa]** | **Qvalue** | **Cscore** | **C3-2** | **C4-2** | **C5-2** | **E2-2** | **E3-2** | |
| A0A0G2K6J1 | DENN domain-containing 1A | Dennd1a | 120.4645 | 1.23E-22 | 0.787164 | -2.19851 | -1.12243 | -2.32555 | -2.58972 | -2.61238 | - |
| F1M805 | UTP6 small subunit processome component | Utp6 | 70.23084 | 3.97E-28 | 0.795101 | -3.04151 | -3.93692 | -1.67742 | -3.3605 | -2.13941 | - |
| A0A096P6L8 | Fibronectin | Fn1 | 272.4461 | 2.66E-75 | 0.843369 | 0.289827 | 2.646698 | 3.939096 | 0.734381 | 1.124997 | |
| A0A0G2JSX1 | Non-structural maintenance of chromosomes element 1 homolog | Nsmce1 | 33.30919 | 0.000604 | 0.737861 | 1.070106 | 0.611183 | 0.106892 | 0.576944 | 0.215285 | |
| A0A0G2K919 | LIM domain only 4 | Lmo4 | 18.0077 | 0.000991 | 0.729988 | -2.47686 | -2.49854 | -3.24286 | -2.17676 | -3.02981 | - |
| B0BNN3 | Carbonic anhydrase 1 | Ca1 | 28.29963 | 9.47E-59 | 0.82902 | 2.366539 | -0.81989 | 3.750589 | 0.768434 | 1.807509 | |
| D3ZVB7 | Mimecan | Ogn | 34.06928 | 7.18E-42 | 0.812023 | -1.11086 | 0.137761 | 0.377591 | 2.075826 | 3.631153 | |
| D4A5T8 | Periplakin | Ppl | 204.1472 | 2.45E-63 | 0.833184 | -0.66663 | 0.434097 | -0.93671 | 0.017401 | 0.217498 | - |
| P07861 | Neprilysin | Mme | 85.79512 | 7.84E-61 | 0.830929 | -0.68506 | -1.03119 | -0.46674 | -1.17448 | -0.54597 | - |
| P70705 | Copper-transporting ATPase 1 | Atp7a | 162.0929 | 6.54E-19 | 0.781901 | -0.49372 | -0.37961 | -0.54716 | -0.41664 | -0.02699 | - |

**Note:** C Table Notes: C is sham group, E is diabetic retina injury group, D is berberine administration group

**Comparison of expression level of key genes**

The expression level of 10 shared genes were analyzed by proteomics and shown in **Figure 4**. The Dennd1a and Utp6 were downregulated in the DM group, while Atp7a, Ppl, Ogn, Nsmcel, Mme, Lmo4, Ca1 and Fn1 were upregulated. However, in the BBR group, Dennd1a and Utp6 were upregulated, and Atp7a, Ppl, Ogn, Nsmcel, Mme, Lmo4, Ca1 and Fn1 were downregulated.

### Molecular Docking

As shown, Atp7a, Cal, Dennd1a, Lmo4, Mme, Nsmce1, and Ppl genes were able to form a stable molecular binding pattern with, indicating that these genes had a good binding activity with berberine, however, Fnl, Ogn, and Utp6 genes were not able to dock with berberine **(Figure 5)**.

### PPI network anlysis

A total 353 genes were obtained, including the 10 shraed genes and 343 genes obtained from the PubMed by searching for the keywords ‘diabetic retinopathy’ **(Table 3)**. The PPI network analysis was performed for 353 genes, and interactions were found between 331 genes after screening out duplicates **(Table4, Figure 6)**. There were 5 genes that were associated with the genes queried in PubMed, which were Atp7a, Lmo4, Mme, Ogn and Ppl.

**Table 3**

**List of 353 genes related diabetic retinopathy.**

| List of 353 genes related diabetic retinopathy | | | | | | |
|---|---|---|---|---|---|---|
| Ghrelin | p-AKT | ERK2 | Bcl-2 | IL-1 β | MCP-1 | FoxOs |
| ROS | p-mTOR | HRMECs | HSP | mRNA | JNK | SMP30 |
| ARPE-19 | REDOX | LC3 | MAPKs | Lmo4 | HRPC | Akt |
| IL-1B | *Col12a1* | Pro | hRPEs | microRNA | RBY79 | GSK-3β |
| IL-18 | *Chad* | PRR | HIF-1*α* | miR-139-5p | SO-RB50 | RPE-EMT |
| NLRP3 | *Bgn* | Gyp17 | COX-2 | lncRNA H19 | TNF | PEI-CeO□ |
| VEGF | PPP1CA | NF-κB | VEGF-A | TGF-β | 5-LOX | HA-CeO□ |
| RPE | YAP | SERCA2a | FGF2 | EndMT | IL-1 | STK25 |
| RGC | STZ | PP1 | miR-140-3p | IL-1β | IL-8 | RGCs |
| Muller | HG | AGEs | circsLC16A12 | IL-17A-KO | iNOS | CCK-8 |
| SOD | Gln | PKC | RUNX1 | IL-17A | FGF | Caspase-3 |
| TUNEL | mTORC1 | syndcan-1 | RNS | IL-17RA | IGF-1 | p53 |
| TNF-α | NLR | CD44 | ML-SA1 | IL-17RA-shRNA | GAPDH | LC3-II |
| IL-6 | GCL | syndecan-3 | MCOLN1 | IL-23 | GSH | LC3-I |
| MCP-1 | caspase-1 | RRMECs | TRPML1 | SIRT1/NOCTh1 | CCN1 | p-p53 |
| MDA | CGA | syndecan-2 | rMC1 | AGE-RAGE | GLP-1RA | p53 |
| T2DM | HREC | syndecan-4 | PGC1α | CUR | GLP-1 | CASP8 |
| GHSR | APRE19 | glypican-1 | SOD3 | INS | TCM | CASP9 |
| IL17A | M2 ipRGCs | glypican-3 | CCN1 | CUR + INS | Keap1-Nrf2-ARE | p38αMAPK |
| RMC | M3 ipRGCs | p-ERK | TLR2 | IL-17 | GMFB | c-Jun N |
| IL17RA | M1 | 4-HNE | DPP | ADGRE1 | miR-5195-3p | JNK1 |
| Th17 | AMPK | WIF1 | GLP | C57BL6 | miR-124-3p | ERK1 |
| Act1 | SIRT1 | Wnt | SGLT | CCL2 | CCK-8 | SGK1 |
| TRAF6 | NAD | pdx1 | eGFR | CD45 | G3BP2 | PAC |
| IKK | NADH | MAP1LC3 | RIPK1 | CXCL1 | p38MAPK | Hsa-miR-3184-3p |
| NFκB | LKB1 | SQSTM1 | RIPK3 | DAMPs | Irs2-KO | hsa-miR-24-3p |
| EAAT1 | TLR4 | BECN1 | TNFR1 | DBA/2J | Irs2 | hsa-miR-197-3p |
| Ins2 | MD2 | DAPI | IFNR | HSPGs | TH | BMP4 |
| USA | NOX4 | EdU | TLR | IL6 | miR-195 | MiR-340-5p |
| CA | NADPH | ERG | MLKL | PCO | ChIP | miR-18a-3p |
| Gibco | TAK-242 | FITC | SGLT2i | PDGF | Co-IP | VEGFR2 |
| MD | HMGB-1 | CTRP3 | Cx43 | PDR | LPS | VEGF121 |
| Abcam | TRX1 | Claudin-5 | TGFβ1 | PVD | P38 MAP | eVEGF-38 |
| ZO1 | PGC-1α | Occluding | P2X7R | PVR | FASN | eVEGF-53 |
| GFAP | NRF1 | CRP | ATP | TG | NFAT2 | MAPK |
| GS | TFAM | INS-1 | NRK-52E | TGF1 | p-NF-κB | Rho GTPase |
| SYTO13 | ERK | c-jun | pCD5 | TM | TRPC6 | CDC42 |
| PI | AMPK | Akt | BCX | Th1 | FC-DenseNet | ROCK |
| Nrf2 | MEK | TLR9 | GAP43 | Th2 | MA-FC-DenseNet | VEGF165 |
| AGE | MEK1 | MTOR | NCAM | αSMA | ATG4D | RIPK3 |
| RAGE | MEK2 | EMT | HO-1 | mTOR | miR-125b-5p | circEhmt1 |
| ECM | circRNA | YY1 | L6H21 | miR-15b-5p | Wnt/β | NFIA |
| Keap1 | TXNIP | VEGFA | QRT-PCR | circ_001209 | RT-qPCR | SA-β-gal |

| | | | | | | |
|---|---|---|---|---|---|---|
| NeH2 | circADAM9 | Snail1 | SIRT2 | Glu | GLP1R | P21 |
| HO1 | CARM1 | Smurf2 | SIRT3 | DR | S1PR2 | OGG1 |
| Nox2 | KLF3 | LOX | SIRT4 | IS | JTE-013 | RGC-5 |
| LC3B-II. | Trx-R | CaMKII | SIRT5 | IL-17 | hRVECs | 8-OHdG |
| p62 | FGF-1 | SH-SY5Y | SIRT6 | NFKB | PFK-2 | ICAM-1 |
| PI3K | p38 MAPK | Bax | SIRT7 | STAT-3 | cJun | JNK |
| Dennd1a | Utp6 | Fn1 | Nsmce1 | Lmo4 | Ca1 | Ogn |
| Ppl | Mme | Atp7a | | | | |

**Table 4**

**Related genes in the PPI network**

| Related genes in the PPI network | | | | | | |
|---|---|---|---|---|---|---|
| ADHFE1 | FGF2 | LY96 | ROS1 | YY1 | PRKAB1 | MCL1 |
| AGER | FN1 | MAP1LC3A | RRAD | DECR1 | GCLC | BCL10 |
| AKT1 | FOXM1 | MAP2K1 | RUNX1 | SGK1 | MAPK14 | SDC4 |
| AKT1S1 | G3BP2 | MAP2K2 | SDC2 | MAPK1 | LOX | GLP1R |
| ALOX5 | GABPA | MAP2K7 | SDC3 | CCL2 | SPARC | DENND1A |
| APC | GAP43 | MAPK1 | SDC4 | CRP | GHSR | ALOX5 |
| ARHGAP18 | GAPDH | MAPK14 | SGK1 | TLR2 | SNAI1 | TXN |
| ATG16L1 | GCLC | MAPK3 | SIRT1 | MAPK3 | BMP4 | MAP1LC3A |
| ATG4D | GFAP | MAPK8 | SIRT2 | NOX4 | SIRT2 | IRS2 |
| ATP7A | GH1 | MCL1 | SIRT3 | IL1B | APC | TH |
| BAX | GHSR | MCOLN1 | SIRT4 | FGF2 | YY1 | IL33 |
| BCL10 | GJA1 | MLKL | SIRT5 | ICAM1 | SMURF2 | SOD3 |
| BCL2 | GLP1R | MME | SIRT6 | TP53 | MAP2K2 | SIRT3 |
| BECN1 | GMFB | MTOR | SIRT7 | EGFR | KDR | SQSTM1 |
| BMP4 | GPC1 | NCAM1 | SLC1A3 | IL18 | PIK3CA | AKT1S1 |
| C1QTNF3 | GPC3 | NDUFS1 | SMURF2 | CXCL8 | NRP1 | ATG16L1 |
| CARKD | GSS | NEIL2 | SNAI1 | CASP3 | SOD1 | GAP43 |
| CARM1 | GSTA4 | NELFCD | SOD1 | NLRP3 | CCNA2 | MAPK8 |
| CASP1 | GTF2H1 | NFATC1 | SOD3 | TLR9 | TJP1 | MAP2K7 |
| CASP3 | HMOX1 | NFIA | SPARC | PTGS2 | GJA1 | BCL2 |
| CASP8 | HSP90AA1 | NLRP3 | SQSTM1 | JUN | BAX | CDC42 |
| CASP9 | HTRA1 | NLRX1 | STIM2 | TLR4 | MAP2K1 | CLDN5 |
| CCL2 | ICAM1 | NOS2 | STK11 | CXCL1 | FASN | NFATC1 |
| CCNA2 | IFNAR1 | NOX4 | STK25 | CASP1 | STK11 | CD44 |
| CD180 | IL17A | NRF1 | STUB1 | AKT1 | PPP1CA | PTPRC |
| CD44 | IL17RA | NRP1 | TBX21 | INS | NOS2 | TRAF6 |
| CDC42 | IL18 | NT5C | TFAM | VEGFA | SIRT7 | TMEM206 |
| CEACAM5 | IL1B | OCLN | TG | TNF | CASP9 | NCAM1 |
| CGA | IL23A | OGG1 | TH | IL6 | HSP90AA1 | TXNIP |
| CHRM1 | IL33 | OGN | TJP1 | TFAM | SIRT6 | FGF1 |
| CLDN5 | IL6 | PIK3CA | TLR2 | CYBB | PPL | S1PR2 |
| CNPY3 | INS | PRKAB1 | TLR4 | TNFRSF1A | FOXM1 | GFAP |
| COL12A1 | IRF6 | PRRT2 | TLR9 | KEAP1 | IL17A | SIRT5 |
| CRP | IRS2 | PTGS2 | TNF | TBX21 | ATP7A | WIF1 |
| CXCL1 | ISL1 | PTPRC | TNFRSF1A | SIRT4 | FN1 | ATG4D |
| CXCL8 | JUN | PVRL1 | TP53 | SIRT1 | OCLN | NLRX1 |
| CYBB | KDR | RGN | TRAF3IP2 | HMOX1 | CASP8 | RIPK1 |
| DENND1A | KEAP1 | RIPK1 | TRAF6 | STUB1 | MTOR | RIPK3 |
| DSPP | LOX | RIPK3 | TXN | GAPDH | BECN1 | MCOLN1 |
| DUSP26 | ROS1 | EMR1 | FAM20C | LMO4 | GH1 | NFIA |
| EARS2 | ISL1 | NELFCD | GATB | PRRT2 | RUNX1 | MOK |
| EGFR | GPC1 | IL23A | RRAD | STIM2 | OGN | TRAF3IP2 |
| EMR1 | HTRA1 | IL17RA | NPTN | TAOK2 | TRPC6 | LY96 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ERG | SDC2 | CEACAM5 | G3BP2 | ST3GAL4 | NEIL2 | MLKL |
| EXTL3 | IRF6 | PVRL1 | PDX1 | STK25 | DOCK7 | SLC1A3 |
| FASN | DSPP | SDC3 | NRF1 | YY1AP1 | GSTA4 | RASIP1 |
| FGF1 | GPC3 | MME | RPE | GSS | TG | OGG1 |
| PPA1 | IFNAR1 | | | | | |

### GO enrichment analysis

As shown, the top 10 BPs of GO enrichment analysis involved in berberine on the treatment of DR were as follows: response to oxidative stress, cellular response to chemical stress, cellular response to oxidative stress, response to molecule of bacterial origin, reactive oxygen species metabolic process, activation of protein kinase activity, response to lipopolysaccharide, cellular response to biotic stimulus, regulation of reactive oxygen species metabolic process, and response to reactive oxygen species. The first 10 CCs involved of GO enrichment analysis were raft, membrane microdomain, membrane region, Golgi lumen, focal adhesion, cell-substrate junction, lysosomal lumen, late endosome, endoplasmic reticulum lumen, and endocytic vesicle. The top 10 MFs involved in berberine on the treatment of DR were NAD+ binding, protein Serine/threonine kinase activity, cytokine receptor binding, NAD-dependent protein deacetylase activity, protein serine/threonine/tyrosine kinase activity, MAP kinase activity, NAD binding, receptor ligand activity, signaling receptor activator activity, and protein deacetylase activity. Among them, the most important biological process is Response to oxidative stress, which involves most cell groups including membrane raft, and the most important molecular function is NAD+ binding.

### KEGG signaling pathways analysis

It was shown that the first ten signaling pathways involved in the berberine on the treatment of DR were as follows: lipid and atherosclerosis, salmonella infection, AGE- signaling pathway in diabetic complications, NOD-like receptor signaling pathway, fluid shear stress and atherosclerosis, Kaposi sarcoma-associated herpesvirus infection, toll-like receptor signaling pathway, TNF signaling pathway, shigellosis, and hepatitis B **(Figure 8A)**. The corresponding genes of each signaling pathway were sorted for the first 20 KEGG pathways, and the pathways were classified. The environmental information processing pathway was TNF signaling pathway. The signaling pathways of organismal systems were as follows: Nod-like receptor signaling pathway, Toll-like receptor signaling pathway, and IL-17 signaling pathway. The signaling pathways involved in the human diseases were lipid and atherosclerosis, salmonella infection, AGE-RAGE signaling pathway in diabetic complications, fluid shear stress and atherosclerosis, Kaposi sarcoma-associated herpesvirus infection, shigellosis, hepatitis B, yersinia infection, influenza A, non-alcoholic fatty liver disease, pathogenic Escherichia coli infection, chagas disease, pertussis, proteoglycans in cancer, tuberculosis, and human cytomegalovirus infection.

**Table 5**

**The top 10 KEGG signaling pathways.**

| ID | Description | pvalue | p.adjust | qvalue | Count |
|---|---|---|---|---|---|
| hsa05417 | Lipid and atherosclerosis | 4.53E-23 | 1.09E-20 | 3.96E-21 | 34 |
| hsa05132 | Salmonella infection | 5.52E-22 | 6.62E-20 | 2.41E-20 | 35 |
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications | 1.34E-19 | 1.07E-17 | 3.91E-18 | 23 |
| hsa04621 | NOD-like receptor signaling pathway | 1.40E-18 | 8.42E-17 | 3.07E-17 | 28 |
| hsa05418 | Fluid shear stress and atherosclerosis | 1.82E-18 | 8.75E-17 | 3.19E-17 | 25 |
| hsa05167 | Kaposi sarcoma-associated herpesvirus infection | 6.00E-18 | 2.11E-16 | 7.68E-17 | 28 |
| hsa04620 | Toll-like receptor signaling pathway | 6.16E-18 | 2.11E-16 | 7.68E-17 | 22 |
| hsa04668 | TNF signaling pathway | 3.35E-17 | 1.00E-15 | 3.65E-16 | 22 |
| hsa05131 | Shigellosis | 4.85E-17 | 1.29E-15 | 4.71E-16 | 30 |
| hsa05161 | Hepatitis B | 8.40E-17 | 2.02E-15 | 7.34E-16 | 25 |

## Discussion

In this paper, we established DR rats model to investigated the effects of berberine on the treatment of DR using behavioral experiments, proteomics, PPI network, GO and KEGG analysis. It was shown that there were 2 down-regulated genes, namely Dennd1a and Utp6, and 8 up-regulated genes, namely Atp7a, Ppl, Ogn, Nsmcel, Mme, Lmo4, Ca1 and Fn1 in the DM group, while the opposite was true in BBR group. Molecular docking showed that berberine could form a stable molecular binding pattern with other genes except Fnl, Ogn and Utp6 Then, a total of 331 genes with interaction relationship were analyzed by GO and KEGG analyses. The results suggested that the most important BP, CC, and MF involved in the berberine on the treatment of DR were response to oxidative stress, raft, and NAD+ binding, respectively, and the most important signaling pathway was lipid and atherosclerosis. (Chen, Lin et al. 2021)

Diabetic retinopathy (DR) is a neurovascular disease that can seriously cause blindness (Youngblood, Robinson et al. 2019). To study the protective effect of berberine on DR, we carried out animal experiments, OCT detection and Morris water maze test (Baldan Ramsey and Pittenger 2010). It has been reported that berberine can protect retinal morphology from hyperglycemic injury and reduce glycogen accumulation (Yin, Tan et al. 2021). The results of Morris water maze showed that the learning and cognitive functions of rats with DM treated with berberine were significantly improved, and the thickness of RPE outer nucleus, intima boundary-core layer and external morphology layer was thickened in DR rats treated with berberine.

We sorted out the data after proteomics measurement, and 73 proteins in sham group and 140 proteins in DR-Berberine group were analyzed. There were 10 shared genes were obtained after intersection using Venn diagram, namely Dennd1a, Utp6, Fn1, Nsmce1, Lmo4, Ca1, Ogn, Ppl, Mme and Atp7a. The polymorphism of Dennd1a gene is associated with obesity, and the complications of obesity will occur after diabetic lesions (Kadioglu, Altun et al. 2018). Utp6 is a protein that can participate in ribosomal biogenesis (Champion, Kundrat et al. 2009). Fn1 is the accumulated component of the extracellular matrix and in the case of hyperglycemia, it is also a glycoprotein that is present in plasma and extracellular matrix (Wang, Zhao et al. 2021). Nsmce1 gene has been reported to maintain genome integrity (Gong, Wang et al. 2020). We hypothesized that DR would lead to decreased learning and cognitive function in rats, and Nsmcelc had a regulatory effect on behavioral cognitive function in rats. Lmo4 can proliferate and inhibit apoptosis, oxidative stress and existing inflammatory response, and also reduce the damage of retinal pigment epithelial cells (Shao, Chen et al. 2021). After modeling of DR, the hippocampal CA1 region is extensively damaged (Zhang, Zhang et al. 2015). Ogn is a homeostasis mediator of bone and glucose and also helps regulate glucose metabolism (Lee, Ali et al. 2018). DM leads to elevated Ppl (blood lipids) after eating, and metabolic activation (Mme) cell populations associated with insulin resistance are generated in obese environments. It has been reported that Atp7a has a protective effect on DM against endothelial dysfunction through extracellular superoxide dismutase (Sudhahar, Urao et al. 2013). Differential expression of heat map showed that there were two down-regulated genes in the DM group, namely Dennd1a and Utp6, and 8 up-regulated genes, namely Atp7a, Ppl, Ogn, Nsmce1, Mme, Lmo4, Ca1 and Fn1. In the BBR group, there were 8 down-regulated genes, Atp7a, Ppl, Ogn, Nsmce1, Mme, Lmo4, Ca1 and Fn1, and 2 up-regulated genes, Dennd1a and Utp6, respectively.

Additionally, the 10 shared genes may plays an important role in the protection of berberine on the treatment of DR. We verified molecular docking with these 10 genes as receptors and berberine as ligands, and all the other 8 genes, Dennd1a, Utp6, Nsmce1, Lmo4, Ca1, Ppl, Mme and Atp7a, could dock with the drug except for Fnl and Ogn target proteins which failed to dock successfully with berberine ligands. Therefore, berberine could improve DR on the basis of the regulation of these genes.

Among the 10 proteomic genes analyzed, Atp7a, Lmo4, Mme, Ogn and Ppl were correlated with the genes queried in PubMed. However, according to the comprehensive score (Zhang, Han et al. 2021), the top 10 genes with the closest association were AKT1:HSP90AA1, AKT1:MTOR, AKT1S1:MTOR, BCL2:BECN1, CASP1:IL1B, CASP1:NLRP3, CASP8:TNFRSF1A, CASP8:RIPK3, CASP8:RIPK1, EGFR:HSP90AA1. These closely related genes may play a key role in the protection of berberine on the DR.

GO enrichment analysis shows that the most important biological process is response to oxidative stress. It has been reported that antioxidant induced by oxidative stress can improve glucose homeostasis, which plays a key role in the pathophysiology of diabetes (Yaribeygi, Sathyapalan et al. 2020). The most involved cell component is membrane raft, the microregion of plasma membrane, which can provide regulation of cell metabolism at different levels (Sviridov and Miller 2020). The most important molecular function is NAD+ binding and NAD plays an important role in cellular energy homeostasis (Chen, Bang et al. 2015). NAD can effectively intervene in diet, so as to control the pathological changes in DM (Yoshino, Mills et al. 2011).

According to the results of GO enrichment analysis, multiple genes regulate each of the first 10 pathways in BP, CC and MF, which may play a role in the protective mechanism of berberine on DR. KEGG pathway analysis showed that the first pathway was lipid and atherosclerosis, and hyperglycemia was through the deficiency of insulin secretion. Insulin is a metabolic hormone and lack of insulin leads to abnormal lipid metabolism. DM induces atherosclerosis, and DM is followed by increased blood glucose levels and dyslipidemia, and abnormal blood lipids (Poznyak, Grechko et al. 2020). Salmonella neck abscess may lead to DM, which is a risk factor for salmonella infection (Pastagia and Jenkins 2013). The third pathway is AGE-RAGE signaling pathway in diabetic complications. AGE is the end product of advanced glycation and RAGE is a transmembrane protein, and RAGE is upregulated with the increase of circulating AGE level. AGE-RAGE signal transduction can also indirectly affect the occurrence of diabetes complications (Kay, Simpson et al. 2016). NOD-like receptor signaling pathway has been identified as a key regulator of inflammation-related angiogenesis (Liu, Lu et al. 2019). The fifth pathway is fluid shear stress and atherosclerosis, which is a regulatory factor of gene expression in vascular cells that is correlated with diabetes abnormalities (Papadaki, Eskin et al. 1999). The six pathway is Kaposi sarcoma-associated herpesvirus infection, (Ye, Zeng et al. 2016). Toll-like receptor signaling pathway is one of the factors of obesity-induced inflammatory response (Rogero and Calder 2018). In the DR, the TNF molecular pathway may be the cause of its pathogenesis and progression (Dewanjee, Das et al. 2018). Shigellosis may be a complication of diabetes. By vaccinating against viral hepatitis, the burden of diabetes is reduced and the risk of developing diabetes is reduced (Karnchanasorn, Ou et al. 2016). There are corresponding enriched genes in each of the signaling pathways of KEGG, and these genes may play a key role in the protective mechanism of berberine on the DR, which needs to be further explored.

## Conclusion

In this study, we explored the key targets and mechanisms of berberine protection against diabetic retinopathy based on proteomics and network pharmacology, and Dennd1a, Utp6, Fn1, Nsmce1, Lmo4, Ca1, Ogn, Ppl, Mme and Atp7a genes were found that they were closely associated with the protective effect of berberine on the diabetic retinopathy.

## Declarations

**Ethical approval and consent for participation**

All procedures were performed in accordance with the guidelines and approval of the Ethics Committee of the Kunming Medical University. Approved by the Animal Experiment Ethics Review Committee of Kunming Medical University, the approval number is KMMU20220852

**Human and animal ethics**

No human studies are involved

The animal ethics code is KMMU20220852

**Public consent**

I declare that all authors agree to publish.

**Whether there is supporting data**

I declare that the data and materials contained in this manuscript have not been published elsewhere and are available.

**Conflict of interest**

There is no conflict interest in this study

**Money**

Translational study of microrNA-target gene regulatory network in stroke and acute brain injury. Major Science and Technology Project of Sichuan Province (in the field of social development), Project No. 2020YFS0043, fund 1 million, period 2020.1-2021.12, principal: Wang Tinghua

**Author's Contribution**

WTH and GXL conceived and designed the study. FC and CJL conducted experiments, LN analyzed data and wrote manuscripts. All authors have read and approved the manuscript. The authors point out that all data were generated internally, without the use of paper mills.

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## Figures

**Figure 1**

**Images of OCT fundus in control, DM, and BBR groups.**

**A.** The OCT images of control group. **B.** The OCT images of DM group. **C.** The OCT images of BBR group. (DM group, rats with diabetes mellitus; BBR group, rats with DR were treated with berberine.)

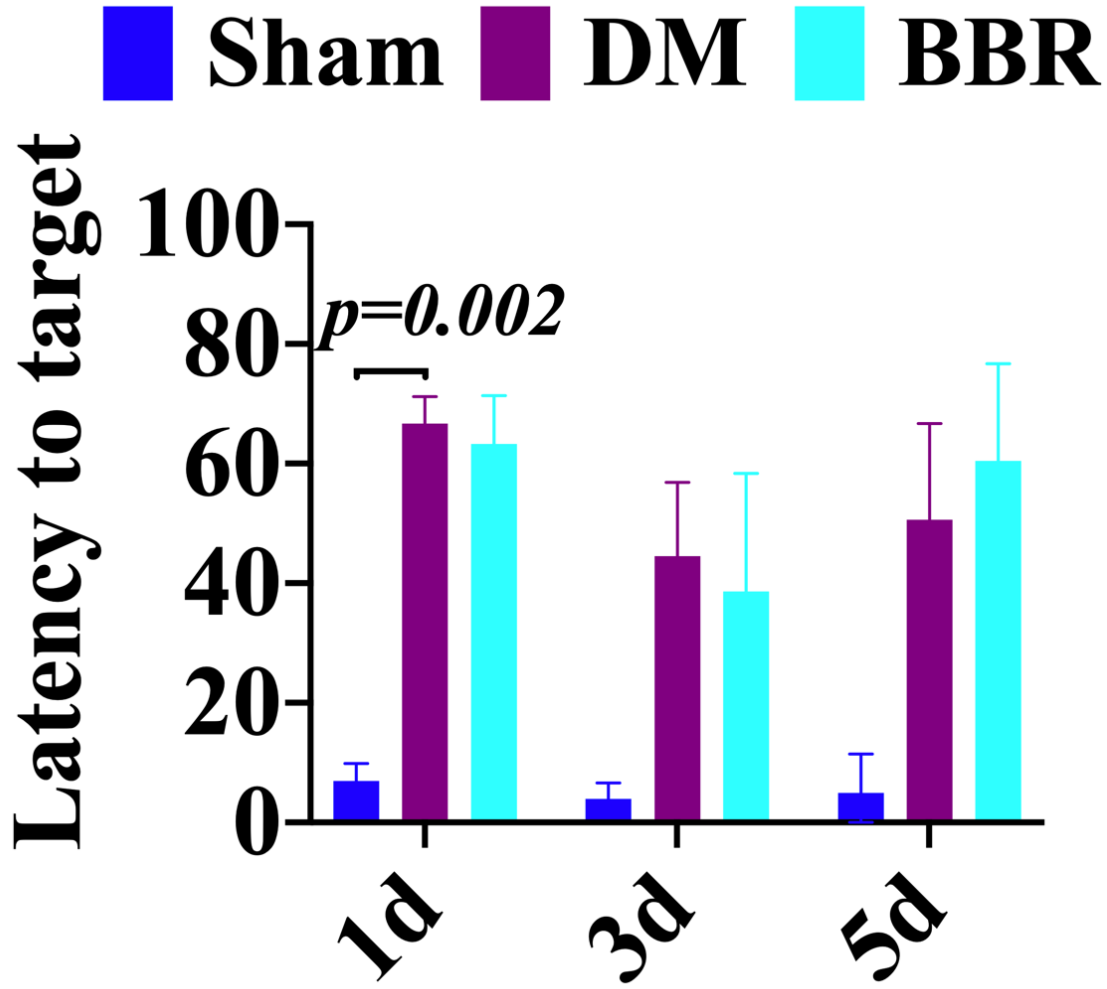


**Figure 2**

**Water maze platform incubation period**

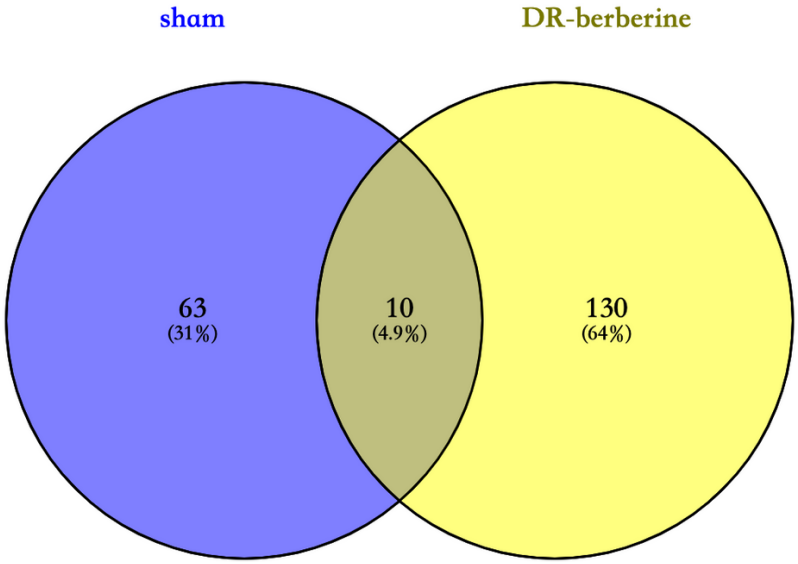


**Figure 3**

**Venn diagram of shared genes between the salidroside and diabetic retinopathy.**

There were 10 shared genes were obtained from the intersection of 73 genes in the Sham group and 140 proteins in DR-Berberine group, including Dennd1a, Utp6, Fn1, Nsmce1, Lmo4, Ca1, Ogn, Ppl, Mme and Atp7a

**Figure 4**

**Expression level of 10 shared genes in each group.**

Red indicates up-regulation of gene expression, and blue indicates down-regulation of gene expression. (Red box indicates up, basket indicates down; C was sham group, E was diabetic retinal injury group,D is berberine drug treatment group

)

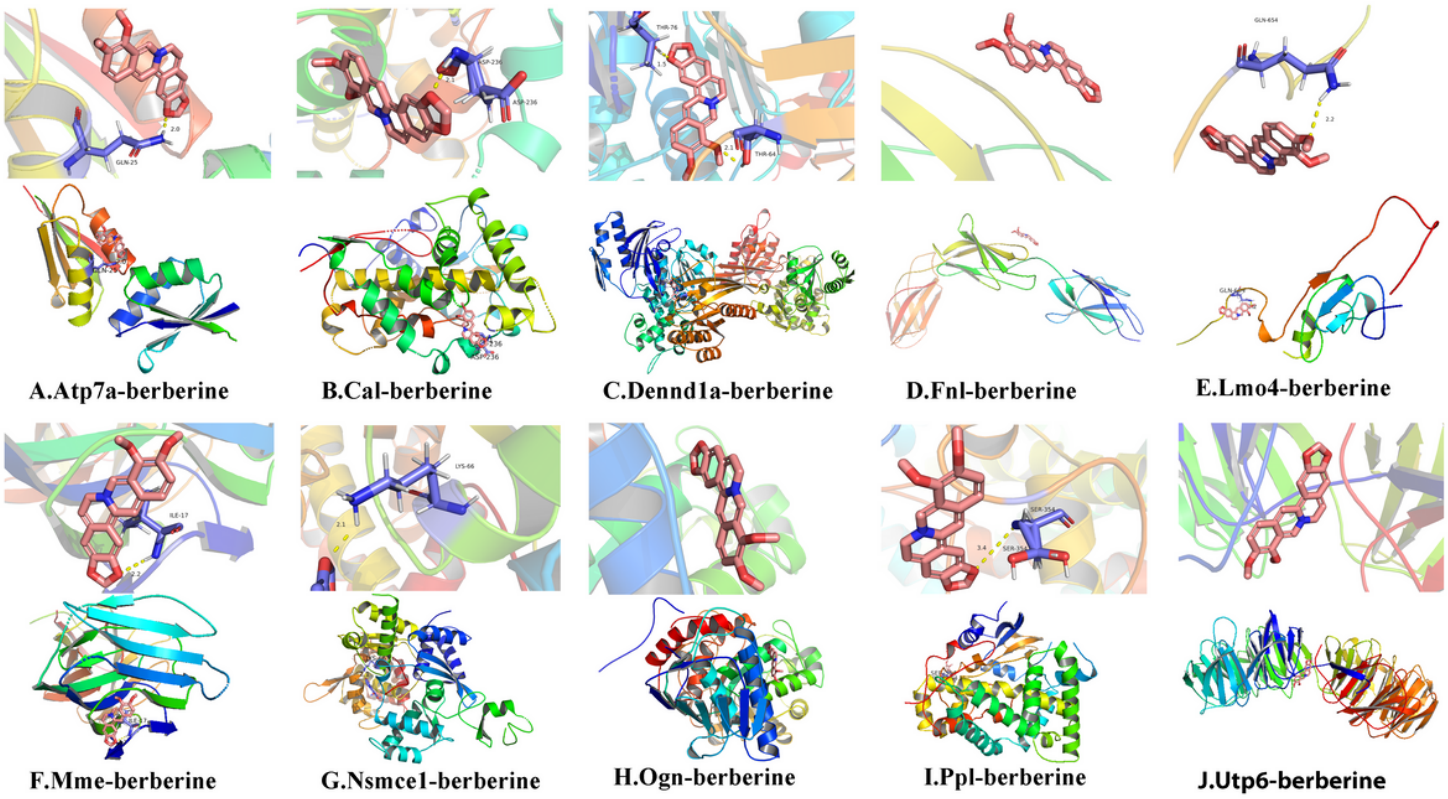


Figure 5

**Molecular docking of 10 shared genes with berberine.**

**A.** Molecular docking of Atp7a with berberine. **B.** Molecular docking of Cal with berberine. **C.** Molecular docking of Dennd1a with berberine. **D.** Molecular docking of Fnl with berberine. **E.** Molecular docking of Lmo4 with berberine. **F.** Molecular docking of Mme with berberine. **G.** Molecular docking of Nsmce1 with berberine. **H.** Molecular docking of Ogn with berberine. **I.** Molecular docking of Ppl with berberine. **J.** Molecular docking of Utp6 with berberine.

Figure 6

**Protein-protein interaction network of genes.**

Figure 7

**GO enrichment analysis**

**A.** Top 20 biological processes (BP) involved in GO analysis. **B.** Top 20 cellular components (CC) involved in GO analysis. **C.** Top 20 molecular function (MF) involved in GO analysis. **D.** The circular diagram of BP, CC, and MF involved in GO analysis.

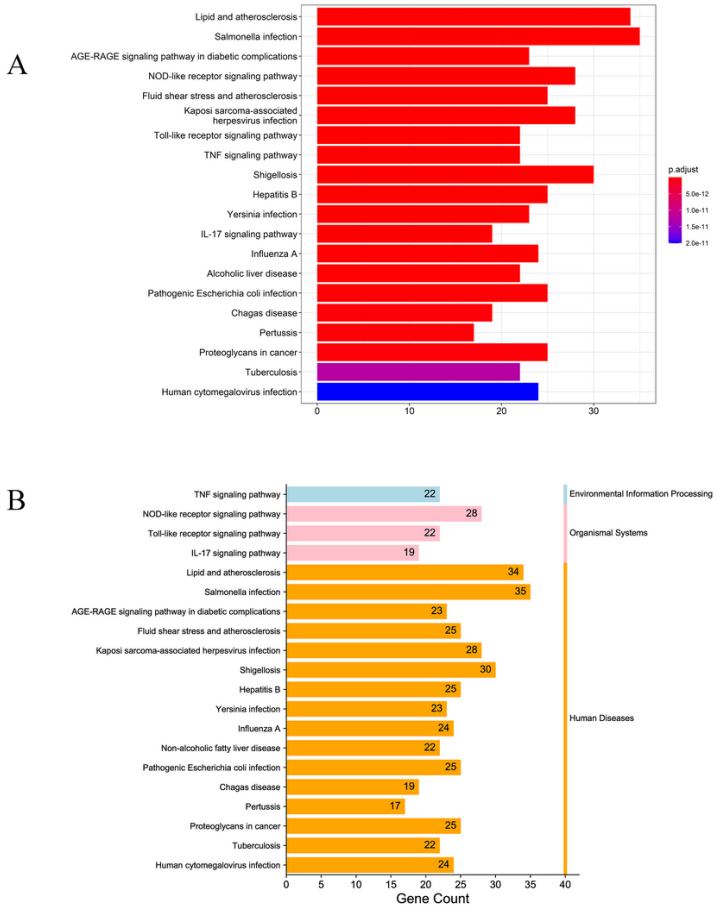


**Figure 8**

**KEGG signaling pathways diagram**

**A.** The top 20 KEGG signaling pathways. **B.** The number of genes involved in the different signaling pathways and systems.