

# Network Pharmacology Study Reveals the Mechanism of Astragali Radix in Treatment of Diabetic Nephropathy

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## Research

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# Abstract

## Background

Diabetic nephropathy (DN), a unique complication of diabetes, could contribute to an increase in mortality. In this study, we predicted and proved the molecular pharmacological mechanism concerning the protective effects of *Astragali Radix* on DN.

## Methods

The same potential target genes from *Astragali Radix* and DN were analyzed and constructed the protein interaction network. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment-related major targets and signal pathways were performed. The drug-ingredients-target-disease network was visually built using Cytoscape 3.6.1. The beneficial pharmacological activities of quercetin from *Astragali Radix* were confirmed by CCK-8 assay, determination of antioxidant parameters and Western blotting analysis.

## Results

There are 12 bioactive components from *Astragali Radix* and 56 same targets between *Astragali Radix* and DN. The GO analysis results showed that the biological processes mainly included protein homodimerization activity. KEGG analysis indicate that the screened targets were most closely linked to the mitogen-activated protein kinase (MAPK) signaling pathway. The drug-ingredients-target-disease network results revealed that the therapeutic effects of *Astragali Radix* mainly included oxidative stress, inflammatory reaction and apoptosis. During the verification process, quercetin from *Astragali Radix* could attenuate cytotoxicity, enhance catalase (CAT) and superoxide dismutase (SOD) activities and suppress MAPK signaling pathway.

## Conclusions

In the current study, network pharmacology with experimental analysis predicted and proved the therapeutic function of *Astragali Radix* by improving antioxidant capacity and suppressing MAPK signaling pathway, these investigations could provide a new perspective for further exploration of *Astragali Radix* on DN treatment.

## Background

Diabetic nephropathy (DN) is a unique complication of diabetes representing main reason of a significant increase in mortality [1]. It is well known that DN is the most prevalent disease resulting in end-stage renal disease (ESRD), in addition its morbidity is significantly rising worldwide [2]. Moreover, the patients with ESRD often need hemodialysis or kidney transplantation to restore the normal kidney function [3]. At present, the therapies for DN mainly focus on controlling the blood pressure and glycemic, and regulating the renin-angiotensin system (RAS) to control the further development of DN [4]. Furthermore, DN is an extremely complicated pathophysiological process, in which multiple biological and pathological processes are involved [5]. Therefore, it is imperative to develop an effective therapies and further understand the pathogenesis for DN.

*Astragali Radix* Bunge (also called as Huangqi in Chinese) has been diffusely utilised as an essential ingredient in over 200 Chinese herb prescriptions to treat weakness, anorexia, chronic fatigue, uterine bleeding, anemia, diarrhea and uterine prolapsed [6, 7]. Currently, pharmacological experiments have showed that *Astragali Radix* possesses a wide variety of biological activities [8], including antioxidant, anti-aging, immunomodulation, anti-inflammation, antitumor, and anti-asthma pharmacological activities [9]. It is especially known to provide obviously protection against kidney, heart, liver, brain, intestine and lung injury [10]. Based on previous report, a derivative of *Astragali Radix* has a therapeutic effect for myocardial injury by ameliorating the myocardial index content [11]. Moreover, *Astragali Radix* has been used as a nutrient supplement or immunomodulatory natural herbal medicine in health food for reinforcing physique and nourishing tissues [12].

Network pharmacology proposed a new and efficient strategies for drug research by selecting potential signal nodes [13]. Moreover, it was aimed to study the function characteristics of drugs in biological systems with a holistic approach [14]. Nowadays, researchers have realized that the “one key, one lock” mode is inadequate to explain the drug effect, particularly in those complex diseases [15]. To explain the complex relationship between drug and diseases by network visualization and large amount of data analysis [16], a series of databases, tools, applications and scripts emerged [17]. Consequently, we analyzed the potential molecular pharmacological mechanism of the roles of *Astragali Radix* for DN through network pharmacology and experimental verification analysis.

## Material And Methods

### Network pharmacology assay

Traditional Chinese Medicine Systems Pharmacology (TCMSP) pharmacology platform (<http://lsp.nwu.edu.cn/tcmsp.php>) was chosen to obtain major ingredients and corresponding targets of *Astragali Radix*. The data of the bioactive ingredient of *Astragali Radix* was gained by screening oral bioavailability (OB)  $\geq$  30% with drug-like (DL) properties  $\geq$  0.18. The genes concerning target were collected through the UniProt database (<https://www.uniprot.org/>). Gene Cards database (<http://www.genecards.org/>) and OMIM database (<http://www.omim.org/>) were utilised to select the targets of DN with “diabetic nephropathy” as the keyword. The Venny online tool (<http://bioinfogp.cnb.csic.es/tools/venny/>) was used to get same target genes between *Astragali Radix* and DN. Protein interaction network was constructed via the String database (<https://string-db.org/>). GO analysis and KEGG enrichment results were performed via DAVID database (<https://david.ncifcrf.gov/>). FDR < 0.05 is used as a screening criterion. The drug-ingredients-target-disease network was formed using Cytoscape 3.6.1 [18].

### Cell Culture

The human renal epithelial HK-2 cell line (ATCC, Shanghai, China), was maintained in DMEM/F12 medium (Gibco, CA, USA) mixing 10% inactivated FBS (Gibco, CA, USA) at 37 °C under 95% air and 5% CO<sub>2</sub>.

### Cck-8 Assay

HK-2 cells were incubated into 96-well plates ( $5 \times 10^4$  cells/well) and divided into control group ( $5.5 \text{ mmol} \cdot \text{L}^{-1}$  glucose), high glucose group ( $30 \text{ mmol} \cdot \text{L}^{-1}$ ), high glucose ( $30 \text{ mmol} \cdot \text{L}^{-1}$ ) +  $50 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$  quercetin group and quercetin group ( $5.5 \text{ mmol} \cdot \text{L}^{-1}$  glucose +  $50 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$  quercetin) for 24 h. Subsequently, CCK-8 solution (Biosharp Life Sciences, China) was supplemented at  $37^\circ\text{C}$  for an hour. Finally, the absorbance results were detected at 450 nm with a multi-well plate reader (Biotek, USA).

## Determination Of Antioxidant Parameters

HK-2 cells were treated with high glucose ( $30 \text{ mmol} \cdot \text{L}^{-1}$ ) and quercetin ( $50 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ ) in 6-well plates for 24 h. Afterwards the HK-2 cells were collected to analyze the antioxidant activities of catalase (CAT) and superoxide dismutase (SOD). The indicators were measured according to the available manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

## Western Blotting Analysis

HK-2 cells were treated with high glucose ( $30 \text{ mmol} \cdot \text{L}^{-1}$ ) and quercetin ( $50 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ ) in 6-well plates for 24 h. HK-2 cells were collected and resuspended with 500  $\mu\text{L}$  cold phosphate buffered solution (PBS) buffer (Gibco, CA, USA). Protein sample concentrations were determined through bicinchoninic acid assay (BCA) protein sample assay kit after cell lysed (Kangwei Century Biotechnology, Beijing, China). Then the experimental protein samples were treated and separated by 6-12% SDS-PAGE electrophoresis and subsequently transferred to activated PVDF membranes. Subsequently, membranes were blocked with 5% skim milk at room temperature (RT) for 2 h and incubated with primer antibodies against P38 MAPK, JNK, phosph-P38 MAPK, phosph-JNK, or  $\beta$ -actin (Cell Signaling Technology, USA) at  $4^\circ\text{C}$  overnight. Next, membranes were correspondingly performed fluorescent secondary antibodies (Cell Signaling Technology, USA) at RT for 2 h. Chemiluminescent protein bands were analyzed by Odyssey Imager (LI-COR, USA). ImageJ 1.41 software (Bethesda, USA) was used to calculate the optical density.

### Statistical analysis

SPSS17.0 statistical software was offered for all data analysis. Values reported were expressed as means  $\pm$  SD. Student's *t*-test and two-way ANOVA were used to analyze significant differences.  $P < 0.05$  was set statistically significant.

## Results

### Drug target prediction

TCMSP database was performed to predict the candidate ingredients and targets from *Astragali Radix*. As shown in Table 1, a total of 12 compounds of *Astragali Radix* were obtained.

Table 1  
Potential effective ingredients of *Astragali Radix*

Molecule ID	Composition	Mr	OB/%	DL	AlogP	Caco-2
MOL000098	quercetin	302.25	46.43	0.28	0.05	1.5
MOL000239	jaranol	314.31	50.83	0.29	0.61	2.09
MOL000296	hederagenin	414.79	36.91	0.75	1.32	8.08
MOL000354	isorhamnetin	316.28	49.6	0.31	0.31	1.76
MOL000371	3,9-di-O-methylnissolin	314.36	53.74	0.48	1.18	2.89
MOL000378	7-O-methylisomucronulatol	316.38	74.69	0.3	1.08	3.38
MOL000380	A1	300.33	64.26	0.42	0.93	2.64
MOL000387	bifendate	418.38	31.1	0.67	0.15	2.56
MOL000392	formononetin	268.28	69.67	0.21	0.78	2.58
MOL000417	calycosin	284.28	47.75	0.24	0.52	2.32
MOL000422	kaempferol	286.25	41.88	0.24	1.77	0.26
MOL000442	1,7-dihydroxy-3,9-dimethoxy pterocarpene	314.31	39.05	0.48	3.11	0.89
A1-(6aR,11aR)-9,10-dimethoxy-6a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol						

## Intersection Target

As a result, quercetin, kaempferol, 7-O-methylisomucronulatol, formononetin, isorhamnetin, hederagenin, 3,9-di-O-methylnissolin, A1, calycosin, jaranol, bifendate and 1,7-dihydroxy-3,9-dimethoxy pterocarpene in *Astragali Radix* have 156, 63, 45, 39, 37, 24, 23, 22, 22, 13, 7 and 4 targets respectively from the Drugbank database. 95 targets for the effective ingredients of *Astragali Radix* were obtained after deleting the duplicate targets. Data mentioned above indicates that the effective ingredients of *Astragali Radix* have multiple targets. Moreover, We speculated that quercetin of *Astragali Radix* was the most likely natural active ingredient to treat DN. By searching the keyword “diabetic nephropathy” in the Gene Cards and OMIM disease database, 2418 disease targets were obtained. As shown in Fig. 1, 56 same targets between *Astragali Radix* and DN in totally are obtained. And specific information is described in Table 2.

Table 2  
Potential targets of *Astragali Radix* for treatment of DN

No.	Gene name	Protein name	UniProt ID	No.	Gene name	Protein name	UniProt ID
1	PTGS1	Prostaglandin G/H synthase 1	P23219	29	GSTM1	Glutathione S-transferase Mu 1	P09488
2	AR	Androgen receptor	Q13771	30	EGFR	Epidermal growth factor receptor	P00533
3	ESR2	Estrogen receptor beta	Q92731	31	VEGFA	Vascular endothelial growth factor A	P15692
4	PRSS1	Trypsin-1	P07477	32	CCND1	G1/S-specific cyclin-D1	P24385
5	CHRM2	Muscarinic acetylcholine receptor M2	P08172	33	PPARG	Peroxisome proliferator activated receptor gamma	P37231
6	ADH1B	Alcohol dehydrogenase 1B	P00325	34	CASP9	Caspase-9	P55211
7	ESR1	Estrogen receptor	Q5T5H8	35	PLAU	Urokinase-type plasminogen activator	P00749
8	FOS	Proto-oncogene c-Fos	P01100	36	RB1	Retinoblastoma-associated protein	P06400
9	AKR1B1	Aldose reductase	P15121	37	IL6	Interleukin-6	P05231
10	F7	Coagulation factor VII	P08709	38	ELK1	ETS domain-containing protein Elk-1	P19419
11	ACHE	Acetylcholinesterase	P22303	39	CASP8	Caspase-8	Q14790
12	RELA	Transcription factor p65	Q04206	40	RAF1	RAF proto-oncogene serine/threonine-protein kinase	P04049
13	OLR1	Oxidized low-density lipoprotein receptor 1	P78380	41	SLC2A4	Solute carrier family 2, facilitated glucose transporter member 4	P14672
14	BCL2	Apoptosis regulator Bcl-2	P10415	42	HIF1A	Hypoxia-inducible factor 1-alpha	Q16665
15	CASP3	Caspase-3	P42574	43	ERBB2	Receptor tyrosine-protein kinase erbB-2	P04626
16	MAPK8	Mitogen-activated protein kinase 8	P45983	44	CAV1	Caveolin-1	Q03135
17	CYP3A4	Cytochrome P450 3A4	P08684	45	MYC	Myc proto-oncogene protein	P01106
18	CYP1A1	Cytochrome P450 1A1	P04798	46	DUOX2	Dual oxidase 2	Q9NRD8
19	ICAM1	Intercellular adhesion molecule 1	P05362	47	NOS3	Nitric oxide synthase, endothelial	P29474

No.	Gene name	Protein name	UniProt ID	No.	Gene name	Protein name	UniProt ID
20	SELE	E-selectin	P16581	48	HSPB1	Heat shock protein beta-1	P04792
21	VCAM1	Vascular cell adhesion protein 1	P19320	49	MGAM	Maltase-glucoamylase, intestinal	O43451
22	CYP1B1	Cytochrome P450 1B1	Q16678	50	CCNB1	G2/mitotic-specific cyclin-B1	P14635
23	ALOX5	Arachidonate 5-lipoxygenase	P09917	51	NFE2L2	Nuclear factor erythroid 2-related factor 2	Q16236
24	GSTP1	Glutathione S-transferase P	P09211	52	NQO1	NAD(P)H dehydrogenase [quinone] 1	P15559
25	AHR	Aryl hydrocarbon receptor	P35869	53	PARP1	Poly [ADP-ribose] polymerase 1	Q5VX85
26	PRKCA	Protein kinase C alpha type	P17252	54	COL3A1	Collagen alpha-1(III) chain	P02461
27	CRP	C-reactive protein	P02741	55	IGFBP3	Insulin-like growth factor-binding protein 3	P17936
28	IGF2	Insulin-like growth factor II	P01344	56	PON1	Serum paraoxonase/arylesterase 1	P27169

## Protein interaction network analysis

As shown in Fig. 2, the network is interrelated among the 56 targets. Moreover, it shows that 56 targets play an important role in DN through multi-channel and multi-directional coordination.

## Go Analysis

We entered 56 same target genes, further, “person” was selected as the species, yielding 20 enrichment results to the DAVID database. The ggplot2.R package (3.2.0 Version) was ready for visualization. The biological processes mainly include protein homodimerization activity, proximal promoter sequence-specific DNA binding, DNA-binding transcription activator activity and cofactor binding. As shown in Fig. 3, the size of the dots represents the number of genes included, and the color changes from blue to red indicates a gradual increase in significance. The protein homodimerization activity contains 11 genes and the gene rate is 19.64%. Therefore, this may become a potential research direction of *Astragali Radix* for treatment of DN.

## Kegg Pathway Analysis

Furthermore, we further imported 56 same target genes, “person” was also selected as the species, producing 13 enrichment results. The enrichment of results was shown in Fig. 4. The potential targets network of *Astragali Radix* for treatment of DN is mainly related to MAPK, HIF, p53 and NF- $\kappa$ B signaling pathway. Results indicated that the target of *Astragali Radix* for treatment of DN was distributed in different metabolic pathways, and multi-component and multi-target interaction may be the mechanism for treating DN. As shown in Fig. 5, the pathway

map of *Astragali Radix* for treatment of DN using KEGG Mapper. Those top-ranking signaling pathways from KEGG analysis were integrated and mapped to obtain the final path map. The 13 targets of *Astragali Radix* for treatment of DN was marked in red, and the proportion was 23.21%. Moreover, the results showed that the targets of *Astragali Radix* for treatment of DN was connected with MAPK, HIF, p53, CASP, Elk1, TNF and NF- $\kappa$ B signaling pathway. It demonstrated that the targets of *Astragali Radix* for treatment of DN was mainly distributed in these signaling pathways, which probably through the interaction of several links to show therapeutic effect.

## Drug-ingredients-target-disease Network

Cytoscape 3.6.1 was utilised to build the drug-ingredients-target-disease network of *Astragali Radix*. The results were shown in Fig. 6. This network contains 70 nodes (1 drug, 12 effective ingredients, 56 targets and 1 disease). The drug, diseases, ingredients and targets respectively used V-shaped, V-shaped, elliptical and rectangular nodes, and the relationship was represented by edges. The more action targets were connected, the greater treatment effect of DN, in which quercetin of *Astragali Radix* may be potential material basis for therapeutic effects. There were 56 targets for *Astragali Radix* for treatment of DN, among which GSTP1, NQO1, NOS3[19] were related to oxidative stress [20], and IL-6 was related to inflammation [21], furthermore, MAPK8, CASP3 and BCL2 were related to apoptosis [22]. Therefore, it is presumed that quercetin of *Astragali Radix* has the most potential to treat DN mainly through inhibiting oxidative stress, inflammatory reaction and apoptosis in the body.

### Quercetin attenuates cytotoxicity in high glucose-induced HK-2 cells

Quercetin from *Astragali Radix* is a potential natural active product for the treatment of DN, which has the chemical structure of ortho phenolic hydroxyl (Fig. 7A). To investigate the effects of quercetin on the cytotoxicity of high glucose-treated renal cells, HK-2 cells were incubated with high glucose (30 mmol·L<sup>-1</sup>) and/or quercetin (50  $\mu$ mol·L<sup>-1</sup>). The experimental result meant that quercetin treatment significantly improved cell viability in high glucose-induced HK-2 cells (Fig. 7B).

### Quercetin enhances antioxidant capacity in high glucose-induced HK-2 cells

The activities of antioxidant-related enzymes may be used as an indicator to reflect the antioxidant status. Therefore, the antioxidant capacity were determined to certify the protective roles of quercetin on high glucose-induced oxidative stress. As shown in Fig. 7C-D, antioxidant parameters SOD and CAT were significantly decreased in high glucose group. On the contrary, quercetin could significantly enhance SOD and CAT activities in high glucose-induced HK-2 cells.

### Quercetin regulates MAPK signaling pathway in high glucose-induced HK-2 cells

In order to further study the molecular mechanisms of quercetin in the treatment of DN, we analyzed the effect of quercetin on MAPK signaling pathway. Western blot analysis results showed high glucose could increase phosphorylation of P38 MAPK and JNK. However, treatment with quercetin significantly suppressed the phosphorylation of P38 MAPK and JNK in MAPK signaling pathway (Fig. 8).

## Discussion



Studies have shown that kidney damage is the most severe in DN, and the damage can aggravate the risk of kidney disease. Importantly, the acute and chronic high glucose state can inhibit the growth of kidney-related cells, further causing cell inflammation, and then induce apoptosis. Network pharmacological method can be useful in providing a global picture of the disease pathogenesis and to identify potential new drug targets for DN. Our results confirmed the evaluation system of DN based on network pharmacology method. Meanwhile, it also revealed that key nodes were selected to get the pathway enrichment by combining GO with KEGG analysis. To explore the correlation between *Astragali Radix* and DN from an integral direction, network pharmacology can offer a novel strategy for in-depth research of TCM due to its holistic and systematic.

Here, TCMSP database could predict the potential effective ingredients and targets from *Astragali Radix*. As shown in Table 1, after deleting the components without corresponding targets, a total of 12 compounds of *Astragali Radix* were found. Upon the analysis data of Gene Cards and OMIM database for DN disease targets, 56 same target genes between *Astragali Radix* and DN could be obtained. As shown in Fig. 1 and Table 2, the data of *Astragali Radix* in the treatment of DN revealed that the same target genes included IL6, RELA, CASP3, CASP8, CASP9, BCL2, MAPK8, NQO1, PTGS1, HIF1A, VCAM1, CYP3A4, CYP1A1, GSTM1 and PPARG, which were involved in many biological processes, such as inflammation, oxidative stress [23], apoptosis, aging [24]. As well documented, researches have certified that inflammation reaction plays a pivotal role in DN [25]. High blood sugar may cause the accumulation of advanced glycation end products, which leads to inflammation in the human kidney cells, increases cell apoptosis, and accelerates the development of DN [26]. Besides, it is reported that the pathogenesis of DN is closely correlated with oxidative stress and apoptosis [27]. The drug protects rats from DN by reducing the expression of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  inflammatory cytokines and inhibiting the oxidative stress response mediated by hyperglycemia [28]. Based on the results above, they indicate that *Astragali Radix* could treat DN by ameliorating these mentioned biological processes. Here, protein interaction networks analysis using the String database indicates that the same targets between *Astragali Radix* and DN are interrelated and play a positive effect in DN through multi-channel and multi-faceted coordination (Fig. 2).

Enrichment analysis showed that the therapeutic effects of *Astragali Radix* mainly involved the biological process of oxidative stress, inflammatory reaction and apoptosis. The signaling pathways concerning *Astragali Radix* for treatment of DN largely related to MAPK, HIF, p53 and NF- $\kappa$ B signaling pathway. These pathways may be the future direction of research in the treatment of DN. GO analysis results displayed that the biological processes included protein homodimerization activity and proximal promoter sequence-specific DNA binding (Fig. 3). The above-mentioned molecular functions were associated with various genes and were significant. So the biological processes may become potential research directions of *Astragali Radix* for treatment of DN. As shown in Figs. 4 and 5, KEGG analysis results were mainly contained p53, MAPK, HIF and NF- $\kappa$ B signaling pathway. More importantly, the potential targets were most closely linked to the MAPK signaling pathway. p53 is a tumor suppressor [29]. It can respond rapidly to genotoxic stresses caused by DNA damage and induce caspase-9 activation and BCL-2 expression, leading to accelerated cell apoptosis [30]. MAPK signaling pathway, including P38 and JNK, mediates cell apoptosis through combining a complex with a proapoptotic factor of p53 [31]. In addition, it can motivate the expression of inflammatory factors mediated through NF- $\kappa$ B signaling pathways and accelerate the kidney pathological changes [32]. HIF-1 was increased in DN mice, which mediates DN-induced kidney fibrotic disease [33]. NF- $\kappa$ B is a key transcription factor regulating inflammatory response in DN. Besides, inflammatory cytokines could stimulate the protein expression of NF- $\kappa$ B signaling pathway [34]. It indicated that the target of *Astragali Radix* for DN treatment was distributed in various metabolic pathways. As shown in Fig. 6, the drug-ingredients-target-disease network of *Astragali Radix* was drawn by Cytoscape software. The results

showed that drugs, ingredients, targets and diseases coordinate with each other. In addition, the treatment of *Astragali Radix* in DN was through the regulation of multi-component, multi-target, and multi-pathway. Meanwhile, its mechanism mainly involved anti-inflammatory, antioxidation and anti-apoptosis.

Although the above results manifested that the molecular pharmacological mechanism of *Astragali Radix* in DN treatment from network pharmacology, there were still some limitations. Then we conducted an experimental verification in the next research. Network pharmacology analysis results prompted quercetin from *Astragali Radix* had the most effective suppression ability against high glucose-induced HK-2 cells owing to ranking first among other ingredients. Therefore, quercetin was chosen as the most representative bioactive ingredient of *Astragali Radix* for further molecular pharmacology experiments. Moreover, KEGG analysis suggested MAPK signaling pathway were the most potential pathway on the treatment of *Astragali Radix* in DN. In this study, quercetin remarkably reduced high glucose-induced cytotoxicity of HK-2 cells (Fig. 7B). Oxidative stress is an obvious trigger for DN, which is induced by continuously accumulation of advanced glycation end metabolites in the kidney tissue [35]. Additionally, antioxidant enzymes can scavenge mass production of reactive oxygen species (ROS) to maintain the balance in redox system [36]. CAT and SOD are essential antioxidant elements, they form a mutually supportive defense system against ROS [37]. Further analysis certified that enhancement of antioxidant ability may be the underlying molecular mechanism by which quercetin inhibited high glucose-induced renal cell apoptosis. In our experiments, quercetin treatment dramatically induced an increase of CAT and SOD activities in high glucose-induced HK-2 cells (Fig. 7C-D). Finally, our results verified the ameliorative role of quercetin in high glucose-induced HK-2 cells by regulating MAPK signaling pathway. In our experiments, quercetin inhibited the phosphorylation of P38 MAPK and JNK, due to significantly reduction of cell apoptosis (Fig. 8).

## Conclusion

Here, we confirmed the positive effect of *Astragali Radix* on DN via network pharmacology. Results showed that *Astragali Radix* could act on multi-target and play a potential therapeutic role on DN by multi-pathway. Molecular mechanism analysis showed that it mainly involved anti-inflammatory, antioxidation and anti-apoptosis. Additionally, experimental analysis demonstrated the therapeutic function of quercetin from *Astragali Radix* by improving antioxidant capacity and suppressing MAPK signaling pathway, which could generate a theoretical foundation for further research of the molecular pharmacological mechanism of *Astragali Radix* in the process of DN treatment.

## Abbreviations

DN

Diabetic nephropathy; TCMSP:Traditional Chinese Medicine Systems Pharmacology; GO:Gene Ontology; KEGG:Kyoto Encyclopedia of Genes and Genomes; DAVID:Database for Annotation, Visualization and Integrated Discovery; OB:oral bioavailability; DL:drug-like; CAT:cytotoxicity, enhance catalase; SOD:superoxide dismutase; ESRD:end-stage renal disease; RAS:renin-angiotensin system; PBS:phosphate buffered solution; BCA:bicinchoninic acid assay; RT:room temperature.

## Declarations

### Authors' contributions

TCM, CX and XDZ conceived and designed the study. TCM, CX and CJB collected the data. TCM performed the data analysis and wrote the manuscript. All authors are responsible for reviewing data. All authors read and approved the final manuscript.

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### **Acknowledgements**

Not applicable.

### **Competing interests**

The authors have declared that there is no conflict of interest.

### **Availability of data and materials**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

TCMSP repository, <http://tcmsp.w.com/tcmsp.php>.

Gene Cards database, <http://www.genecards.org/>.

UniProt database, <https://www.uniprot.org/>.

Venny online tool, <http://bioinfogp.cnb.csic.es/tools/venny/>.

String database, <https://string-db.org/>.

### **Consent for publication**

Not applicable.

### **Ethics approval and consent to participate**

Not applicable.

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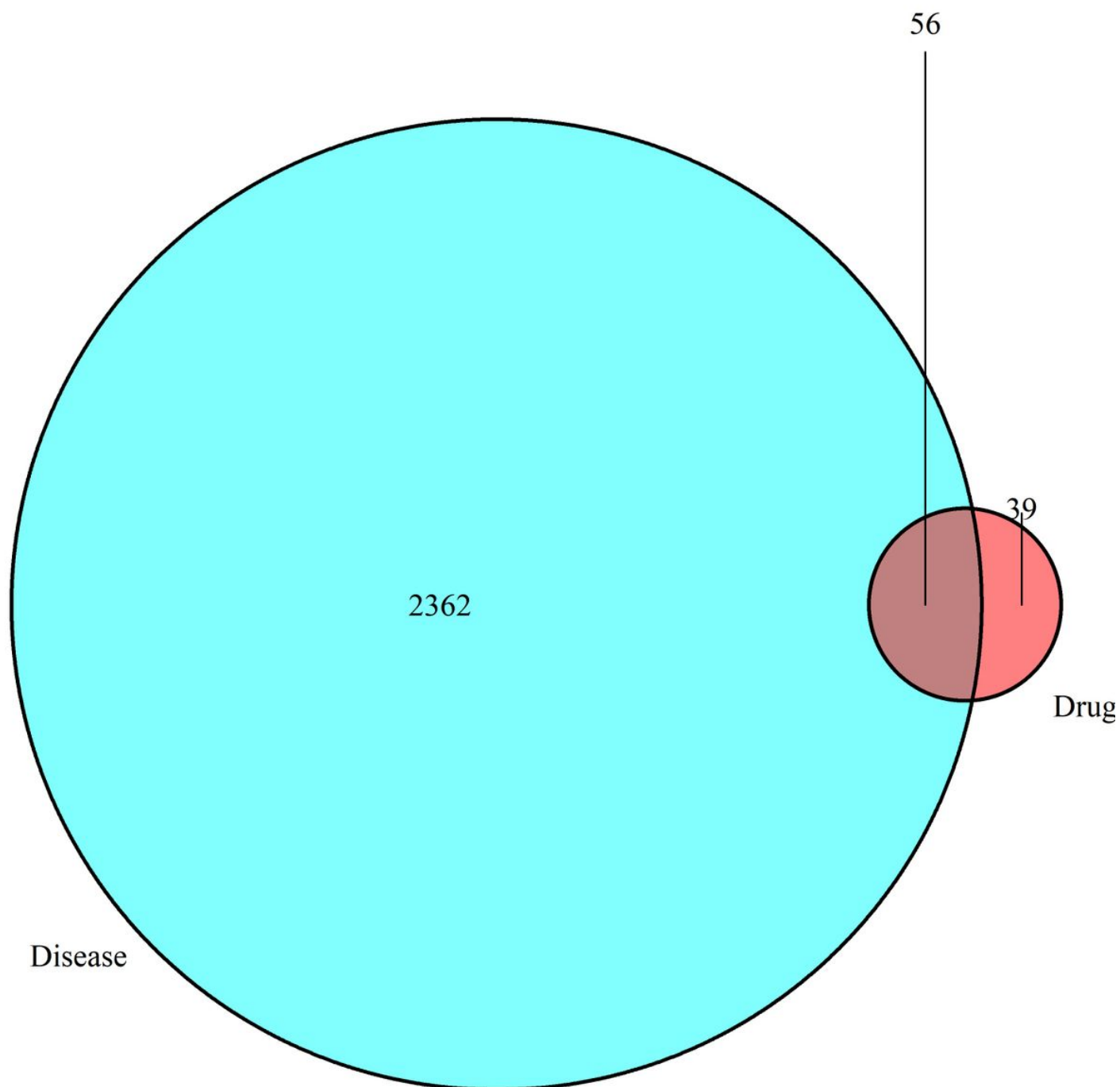
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## Figures

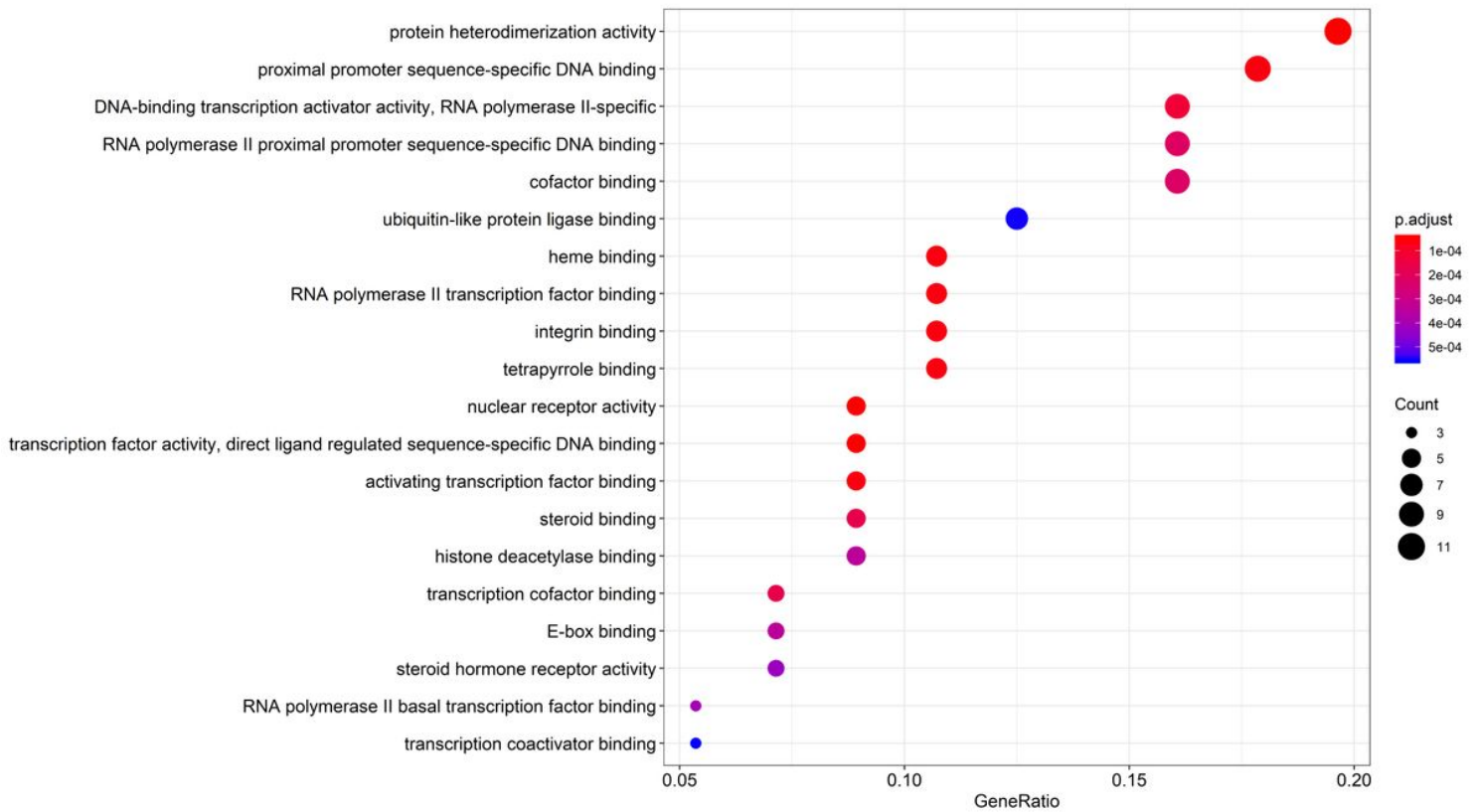


Venn diagram of Astragali Radix and DN targets.



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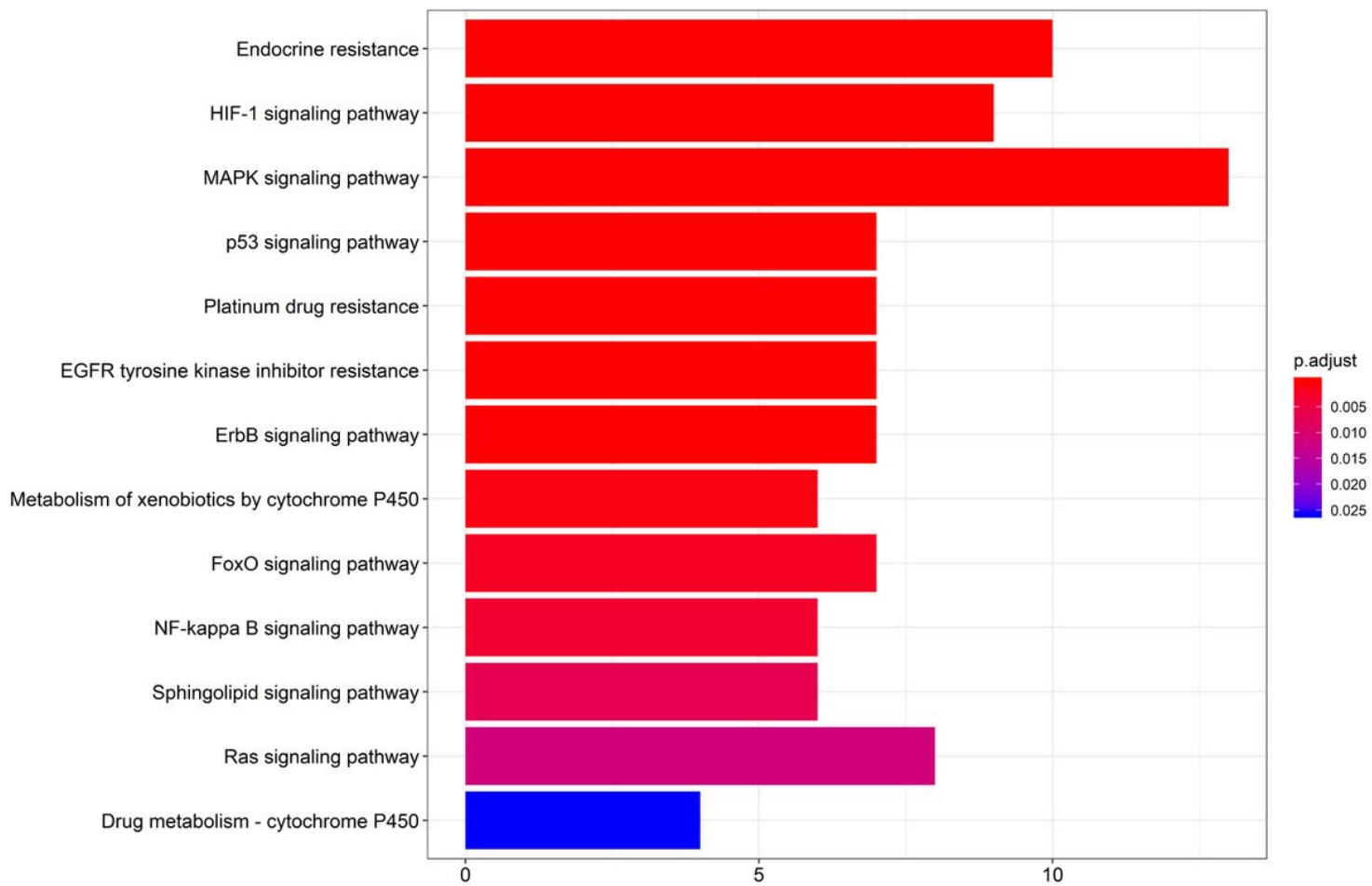




**Figure 3**

GO analysis of potential targets of Astragali Radix for treatment of DN. The ggplot2.R package (3.2.0 Version) was used for visualization. The size of the dots represents the number of genes included, and the color changes from blue to red indicates a gradual increase in significance.





**Figure 4**

Enrich KEGG pathways analysis of potential targets of Astragali Radix for treatment of DN.

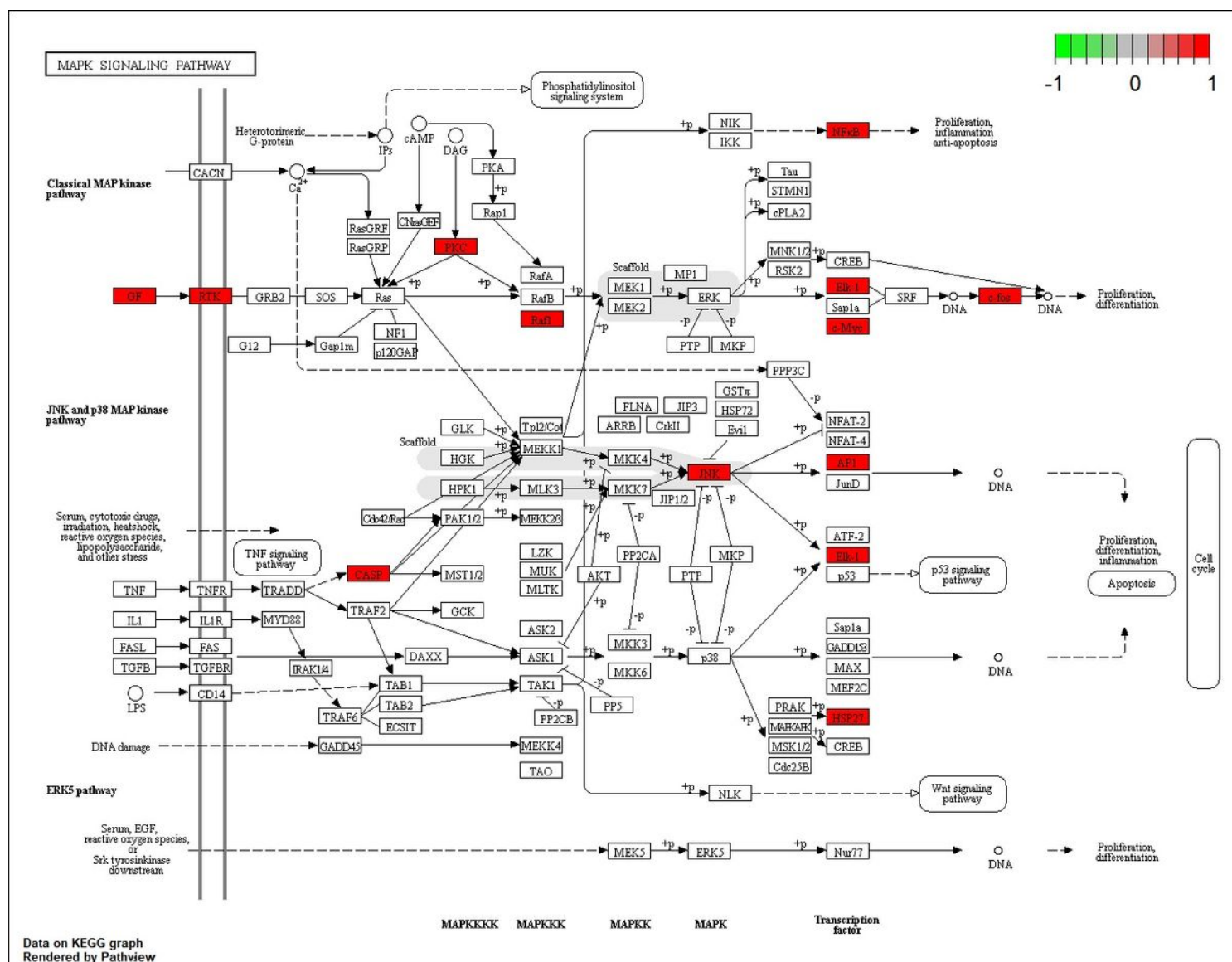
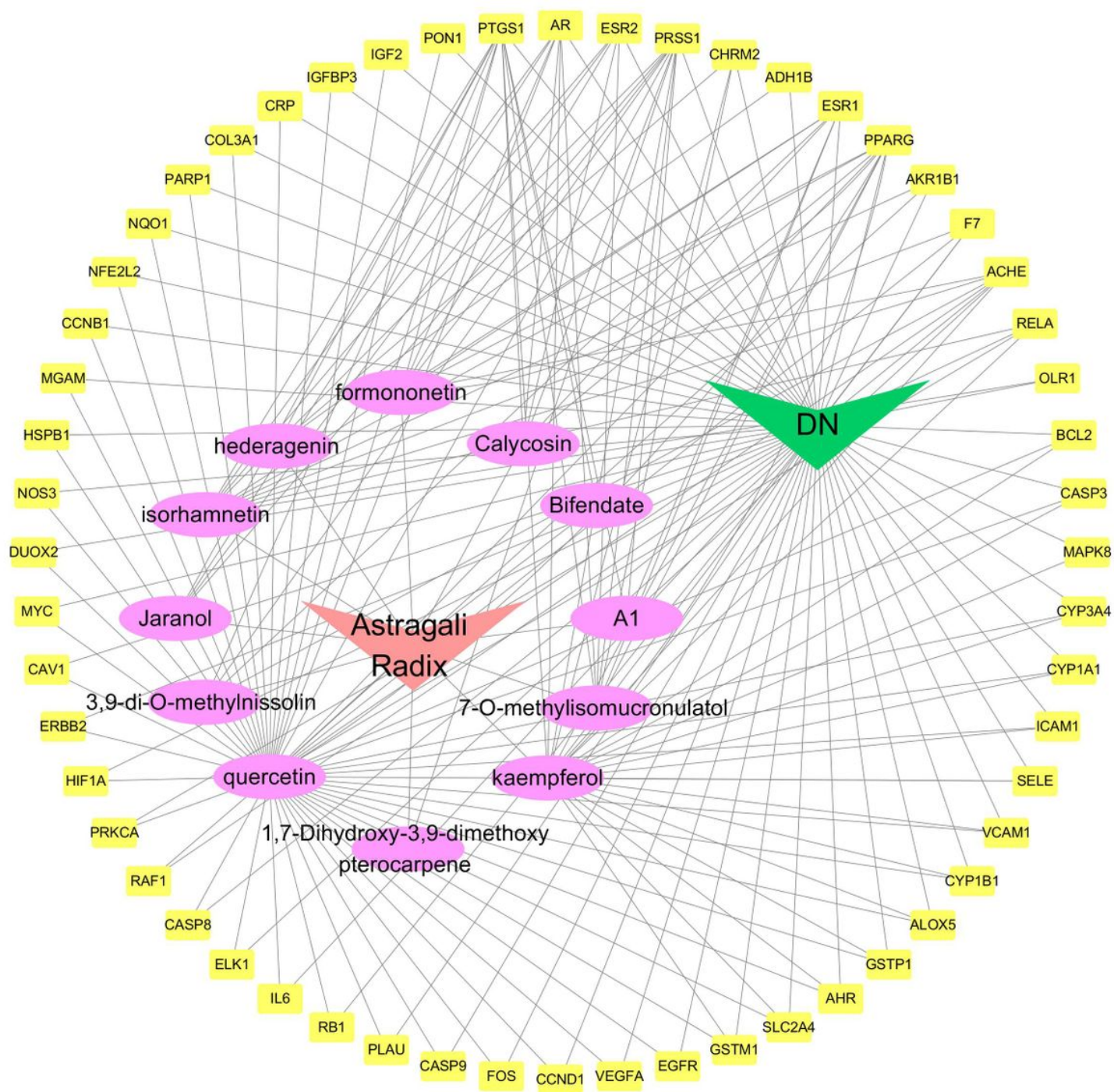


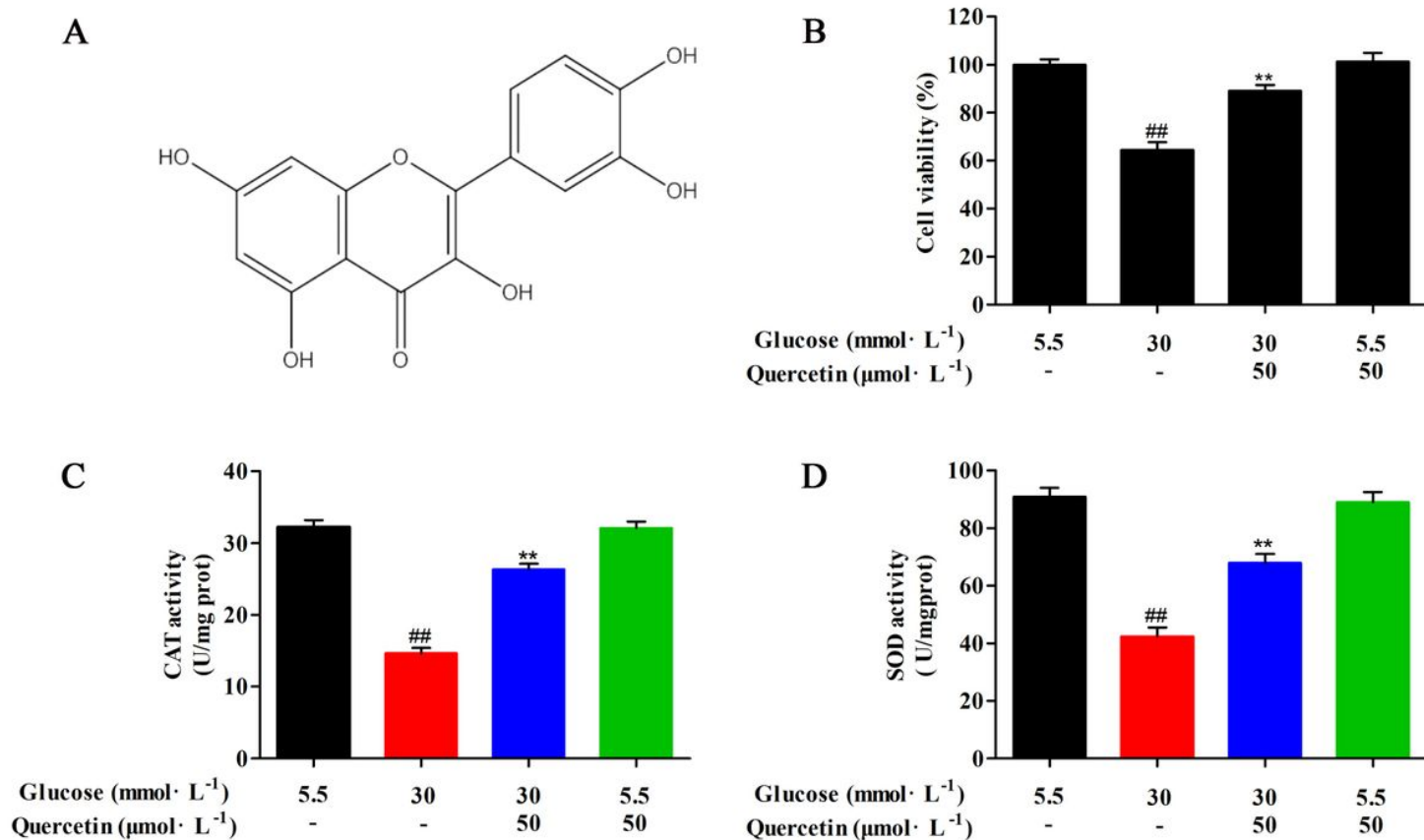
Figure 5

Target of Astragali Radix in DN pathway. The pathway map of Astragali Radix for treatment of DN using KEGG Mapper. Those top-ranking signaling pathways from KEGG analysis were integrated and mapped to obtain the final path map.



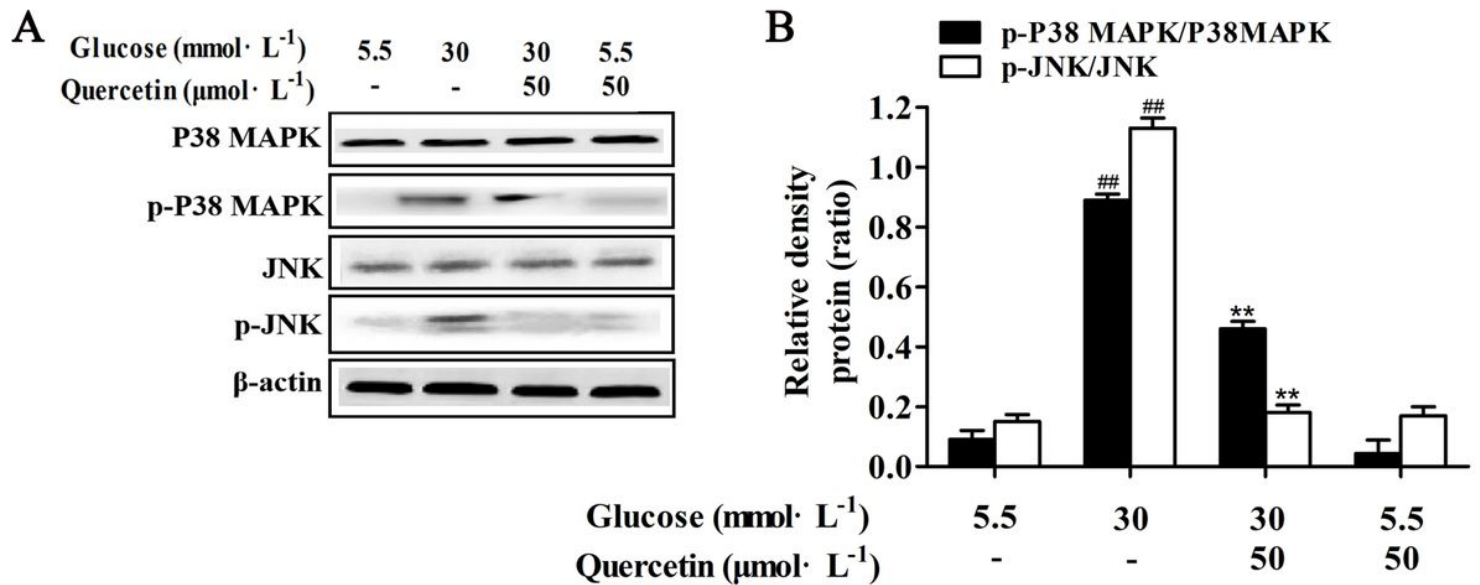
**Figure 6**

Drug-ingredients-targets-diseases network of Astragali Radix for treatment of DN. Cytoscape 3.6.1 was chosen to draw the drug-ingredients-target-disease network. The drug, diseases, ingredients and targets respectively used V-shaped, V-shaped, elliptical and rectangular nodes, and the relationship was represented by edges.



**Figure 7**

Effects of quercetin on the cytotoxicity and antioxidant capacity in high glucose-induced HK-2 cells. (A) Chemical structure of quercetin. Its molecular weight is 302.25. (B) HK-2 cells were divided into control group (5.5 mmol·L<sup>-1</sup> glucose), high glucose group (30 mmol·L<sup>-1</sup>), high glucose (30 mmol·L<sup>-1</sup>) + 50 μmol·L<sup>-1</sup> quercetin group and quercetin group (5.5 mmol·L<sup>-1</sup> glucose + 50 μmol·L<sup>-1</sup> quercetin) for 24 h. Cell viability was measured by a CCK-8 assay. (C-D) HK-2 cells were treated with high glucose (30 mmol·L<sup>-1</sup>) and quercetin (50 μmol·L<sup>-1</sup>) in 6-well plates for 24 h. Effects of quercetin on CAT and SOD activities. Values were expressed as mean ± SD (n=6). <sup>##</sup>P<0.01 vs control group, <sup>\*\*</sup>P<0.01 vs high glucose group.



**Figure 8**

Quercetin suppressed MAPK signaling pathway in high glucose-induced HK-2 cells. HK-2 cells were treated with high glucose (30 mmol·L<sup>-1</sup>) and quercetin (50 μmol·L<sup>-1</sup>) in 6-well plates for 24 h. (A) The protein expressions of P38 MAPK, JNK, phospho-P38 MAPK and phospho-JNK were measured by Western blotting. (B) Quantification and statistical analysis of MAPK activation. Values were expressed as mean ± SD (n=3). ##P<0.01 vs control group, \*\*P<0.01 vs high glucose group.