Tropical Journal of Pharmaceutical Research September 2023; 22 (9): 1815-1822
**ISSN:** 1596-5996 (print); 1596-9827 (electronic)


Available online at http://www.tjpr.org
**http://dx.doi.org/10.4314/tjpr.v22i9.7**

**Original Research Article**

# Investigation of the mechanism of *Astragalus membranaceus* in the treatment of lumbar disc herniation using network pharmacology

**WenJie Ke[1], Chengwei Yu[2]*, Wei Liu[3], Haifeng Liu[2]**
*[1]Department of Nursing, [2]Department of Orthopedics, [3]Department of Rehabilitation, Wenzhou TCM Hospital Affiliated to Zhejiang Chinese Medical University, Wenzhou, Zhejiang Province 325000, China*

**For correspondence:* ***Email:*** *yuchengwei3050901@163.com;* ***Tel:*** *+86-15990703003*

*Sent for review: 16 May 2023* *Revised accepted: 27 August 2023*

## Abstract

***Purpose:*** *To determine the underlying mechanisms of action of Astragalus membranaceus in lumbar disc herniation (LDH) treatment.*
***Methods:*** *This study utilized network pharmacology analysis and STRING database to identify compound targets and visualize PPI network. Furthermore, an LDH model was induced in human nucleus pulposus cells using lipopolysaccharide (LPS), and then the vital target genes were evaluated in this model treated with active components of Astragalus membranaceus.*
***Results:*** *Network pharmacology analysis indicates that several key proteins, including vascular endothelial growth factor A (VEGF A), AKT1, JUN, prostaglandin-endoperoxide synthase 2 (PTGS2), interleukin-6 (IL-6), matrix metallopeptidase 9 (MMP9), interleukin-1β (IL-1β), C-X-C motif chemokine ligand 8 (CXCL8), epidermal growth factor (EGF) and matrix metallopeptidase 2 (MMP2) may play essential roles in LDH treated with Astragalus membranaceus. The active components in Astragalus membranaceus suppressed the production of IL-1β and IL-6, and increased the expressions of VEGF A, MMP9 and MMP2 in LPS-induced LDH model.*
***Conclusion:*** *The active components of Astragalus membranaceus effectively inhibits inflammation in LPS-induced LDH model, indicating that Astragalus membranaceus is a potential therapeutic candidate for LDH treatment.*

***Keywords:*** *Astragalus membranaceus, Inflammation, Lumbar disc herniation, Network pharmacology*



**Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts**

## INTRODUCTION

Lumbar Disc Herniation (LDH) is a clinical syndrome arising from the partial or complete rupture of the annulus fibrosus in the lumbar intervertebral disc. This rupture can occur due to diverse factors, leading to the posterior protrusion of the nucleus pulposus protrudes. Consequently, this protrusion results in irritation or compression of the nerve roots and cauda equina [1]. Patients with LDH exhibit diverse clinical manifestations such as low back pain, numbness, and weakness in the lower limbs. And cauda equina nerve damage may occur, leading to defecation disorders and even paralysis. The available approaches for

addressing LDH encompass conservative management and surgical intervention. The conservative treatment is the first choice for LDH. Therefore, it is crucial to develop complementary and alternative therapies for LDH [2].

*Astragalus membranaceus* (AM) boasts an extensive historical legacy, and is employed for clinical purpose in China. Astragalus is effective in invigorating *qi* and raising *yang*, benefiting health and strengthening appearance. It is widely reported in ancient literature that *Astragalus* directly invigorates *kidney qi*. In the Rihuazi Materia Medica, it is documented that *Astragalus* has the ability to "enhance vital energy and fortify bones and muscles". In clinical application, *Astragalus* has been utilized to enhance lumbar bone mineral density, inhibit bone resorption, and maintain favorable balance through regulating bone metabolism and preventing bone loss among individuals with primary osteoporosis. Additionally, it holds potential to ameliorate symptoms of postmenopausal osteoporosis, promote bone formation and curtail bone resorption [3-5].

It is known that traditional Chinese medicine is characterized by multi-component, multi-target and multi-way for diseases treatment. Network pharmacology is a drug-target-gene-disease-based interaction network, including chemo-informatics, bioinformatics, network biology and pharmacology [6]. It has become a widely used tool to systematically unravel the complex network relationship between bioactive components and underlying mechanisms of traditional Chinese medicine from a system perspective [7].

To determine the mechanism of *Astragalus membranaceus* in LDH disease treatment, network pharmacology was conducted in this study, to unravel the vital target genes of *Astragalus membranaceus*, and demonstrate how *Astragalus membranaceus* inhibits inflammation in LPS-induced LDH model.

## EXPERIMENTAL

### Screening of active components and target sites of lumbar disc herniation

The TCMSP database (https://old.tcmsp-e.com/tcmsp.php) was used to screen the bioactive components based on the criteria of oral bioavailability ≥ 30 % and drug-likeness ≥ 0.18. The targets of these components were searched through TCMSP and Swiss target prediction websites (http://www.swisstargetprediction.ch/).

### Collection of *Astragalus membranaceus*-related targets

The disease targets of *Astragalus membranaceus* were surveyed using two public databases: Online Mendelian Inheritance in Man (OMIM, https://omim.org/) database and GeneCards (https://www.genecards.org/) database, with the keywords "Astragalus membranaceus".

### Analysis of drug regulatory network and protein interaction network

The potential targets of components in lumbar disc herniation and *Astragalus membranaceus*-related targets were collected and imported into Cytoscape 3.8.2 software (http://www.cytoscape.org) to establish a network of lumbar disc herniation-ingredients- *Astragalus membranaceus*-target. The overlapping targets of drugs and diseases in the intersection were inputted into STRING database (https://string-db.org/) to construct protein interaction network. Cytoscape 3.8.2 was used to perform visualization and also to construct a protein-protein interaction (PPI) network.

### Functional enrichment analysis

To investigate the biological function and signaling pathways associated with *Astragalus membranaceus*, the gene ontology (GO) analysis and the Kyoto encyclopedia of genes and genomes (KEGG) analysis were employed. KEGG and GO analysis were screened for q values $< 0.05$.

### Molecular docking

For molecular docking of active components of *Astragalus membranaceus* and key genes, the 3D molecular structures of kaempferol, quercetin and formononetin were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/), and the protein structures of some key genes were downloaded through the Protein Data Bank (PDB, https://www.rcsb.org/). The molecular docking was performed using the CB-dock2 online tools (https://cadd.labshare.cn/cb-dock2/php/index.php).

### *Astragalus* inhibits the inflammation of nucleus pulposus cells

Human nucleus pulposus (NP) cells isolated from lumbar disc herniation tissues were treated with LPS to establish an *in vitro* LDH model. Then, NP cells were exposed to kaempferol, quercetin, formononetin and *Huangqi* decoction,

respectively. The inflammatory factors, IL-1β, IL-6, VEGFA, MMP-9 and MMP2, were assessed [8].

### Enzyme-linked immunosorbent assay (ELISA)

The concentrations of IL-1β and IL-6 in the cell medium were evaluated using ELISA kits of IL-1β (ab214025, Abcam) and IL-6 (ab178013, Abcam). A 100 µL sample was introduced into the ELISA well and incubated for 2 h. Then 100 µL detected antibody was added, and the mixture underwent an additional 1 h incubation. Afterward, the wells were subjected to treatment with enzyme working reagent and TMB reagent. The reaction was ultimately terminated by addition of stop solution. The absorbance value was read at 450 nm.

### Western blot assay

The total protein were extracted using lysis buffer (25 mM Tris-HCl pH 7.4, 250 mM NaCl, 50 mM KCl, 10 % glycerol, 0.5 % NP-40). The lysates were subjected to SDS-PAGE gel running and transferred to nitrocellulose membrane followed by incubation with the primary antibodies against VEGF A (19003-1-AP, ProteinTech, IL, USA), MMP9 (27306-2-AP, ProteinTech), MMP2 (10373-2, ProteinTech) and β-actin (20536-1-AP, ProteinTech). After incubation with horseradish peroxidase labeled with anti-rabbit IgG (B900210, ProteinTech). The target bands were visualized with ECL reagents (Solarbio, Beijing, China). The relative intensity of each band was measured using ImageJ software and normalized to β-actin [9].

### Statistical analysis

The data are expressed as mean ± standard deviation (SD). Statistical evaluation was performed by one-way analysis of variance (ANOVA) to compare the means among the groups, using SPSS 25.0 version [10]. $P<0.05$ was taken as indicating statistical significance.

## RESULTS

### Potential targets of LDH and *Astragalus membranaceus*

Based on oral bioavailability ≥ 30 % and drug-likeness ≥ 0.18, 20 bioactive components of *Astragalus membranaceus* were screened using TCMSP database. The targets of these components were searched through TCMSP and Swiss target prediction websites, and a total of 525 targets were acquired (Figure 1 A). The targets of LDH were screened using GeneCards and OMIM databases, resulting in the acquisition of 698 targets. Statistical computing, conducted using the R project, was employed for data screening. Through the intersection of 525 *Astragalus membranaceus*-related targets and the 698 LDH-related targets, 84 shared recognitions were identified. These overlapping targets were designated as the core targets for subsequent investigations (Figure 1 B).

A

| ID | Ingredient |
|---|---|
| MOL000387 | Bifendate |
| MOL000033 | (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol |
| MOL000378 | 8,10-dimethoxypterocarpan-3-O-β-D-glucoside |
| MOL000296 | hederagenin |
| MOL000449 | 7,2'-Dihydroxy-3',5-dimethoxy-isoflavone |
| MOL000374 | 5'-hydroxyiso-muronulatol-2',5'-di-O-glucoside |
| MOL000422 | kaempferol |
| MOL000098 | quercetin |
| MOL000417 | Calycosin |
| MOL000439 | isomucronulatol-7,2'-di-O-glucosiole |
| MOL000354 | isorhamnetin |
| MOL000239 | Jaranol |
| MOL000371 | 3,9-di-O-methylnissolin |
| MOL000211 | Mairin |
| MOL000383 | (3aR,11aR)-9,10-dimethoxy-3a,11a-dihydro-6H-benzofurano[3,2-c]chromen-3-ol |
| MOL000438 | (3R)-3-(2-hydroxy-3,4-dimethoxyphenyl)chroman-7-ol |
| MOL000433 | FA |
| MOL000392 | formononetin |
| MOL000378 | 7-O-methylisomucronulatol |
| MOL000358 | isoflavanone |

B

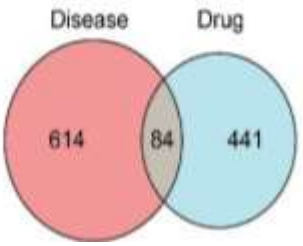



**Figure 1:** Potential targets of LDH and *Astragalus membranaceus*. (A) 20 bioactive ingredients of *Astragalus membranaceus* were screened using TCMSP database; (B) 84 overlapping recognitions were obtained by intersection targets between 525 *Astragalus membranaceus*-related targets and 698 LDH-related targets. LDH: Lumbar disc herniation

### Enrichment analysis of GO and KEGG

To investigate the potential signaling pathway linked to Astragalus membranaceus in the treatment of LDH, GO and KEGG enrichment analysis were applied based on the potential targets. As suggested in the results, the response to endopeptidase activity was identified as the most enriched biological progress by GO functional enrichment analysis (Figures 2 A and B). As revealed by the analysis of overlapping genes via KEGG pathway enrichment analysis, the predominant enriched signaling pathways associated with the treatment of LDH using *Astragalus membranaceus* were the TNF, IL-17

and HIF-1 signaling pathways (Figure 2 C and D).

**PPI network of LDH and *Astragalus membranaceus***

A PPI network was created using the STRING database, which was then visually analyzed using Cytoscape 3.8.2. The edges represent protein-protein and nodes represent proteins. To identify the core proteins of *Astragalus membranaceus* intervention for LDH, Cytoscape software was applied to mine important targets (Figure 3 A). Based on the results from the analysis, a new PPI network was acquired to uncover more critical proteins. Eventually, the final PPI network concentrated on the top 10 targets of Astragalus membranaceus in treating LDH, sorted by degree values. The most prominently valued target nodes include JUN (c-Jun), vascular endothelial growth factor A (VEGF A), AKT1, prostaglandin-endoperoxide synthase 2 (PTGS2), interleukin-6 (IL6), matrix metalloproteinase 9 (MMP9), MMP2, interleukin-1beta (IL-1β), C-X-C motif chemokine ligand 8 (CXCL8) and epidermal growth factor (EGF). These high-value target nodes are likely to play crucial roles in the treatment of LDH using *Astragalus membranaceus* (Figure 3 B).

**Molecular docking of *Astragalus membranaceus* with its key target genes**

The active components of *Astragalus membranaceus* quercetin, kaempferol and formononetin were used for the molecular docking. Their 3D molecular structures were downloaded from PubChem. The key target genes contain VEGFA, IL-6, IL-1β, MMP9 and MMP2, and their protein structures were downloaded from the Protein Data Bank. The molecular docking was performed using CB-dock2. VEGFA, IL-6, IL-1β, MMP9 and MMP2 were successfully performed for molecular docking with each active component (Figures 4 A - E), and the vina scores were added to the table (Figure 4 F). The data suggested that *Astragalus membranaceus* binds with the predicted key target genes.

**Astragalus inhibited inflammation in LDH model**

Given that *Astragalus membranaceus* bound to VEGFA, IL-6, IL-1β, MMP9 and MMP2 which are typical molecules involved in inflammation, the *in vitro* LDH model was established to study the effect of the inhibition by *Astragalus on inflammation*. The data from ELISA revealed that IL-1β and IL-6 levels were significantly decreased in groups treated with quercetin, kaempferol, formononetin and huangqi decoction (Figure 5 A). Moreover, western blots data showed that the expression of VEGFA, MMP-9 and MMP2 were significantly increased when the cells were treated with quercetin, kaempferol, formononetin and huangqi decoction (Figure 5 B). Therefore, *Astragalus* suppressed the inflammatory activity in LPS-induced nucleus pulposus cells.

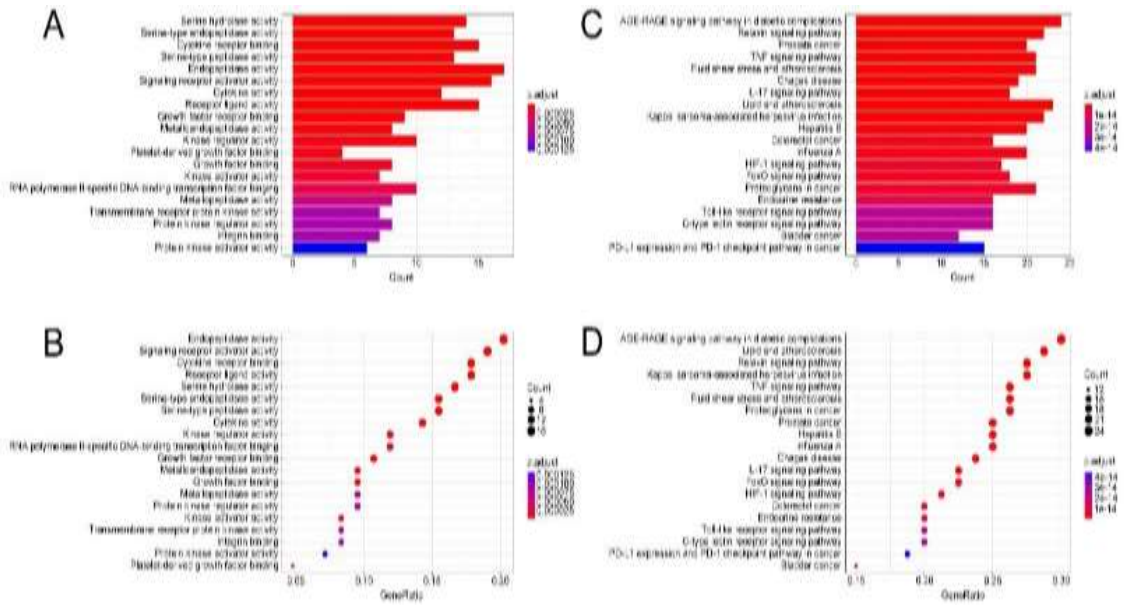



**Figure 2:** Enrichment analysis of GO and KEGG. (A) Bar plot diagram of GO analysis for mainly biological pathways; (B) Dot plot diagram of GO analysis for mainly biological pathways; (C) Bar plot diagram of KEGG enrichment analysis of the mainly enriched genes; (D) Dot plot diagram of KEGG enrichment analysis of the mainly enriched genes

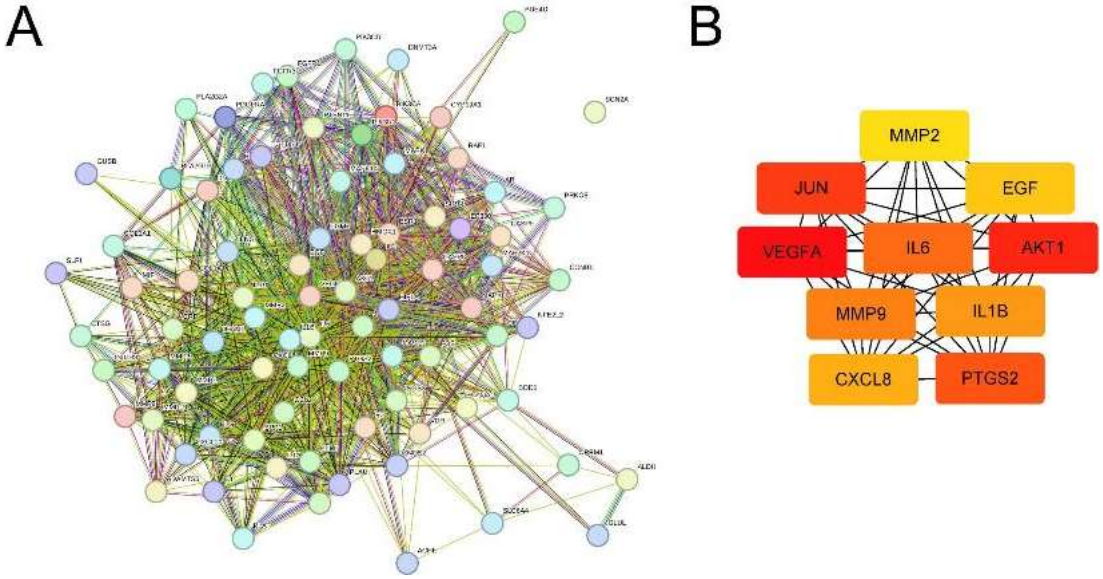
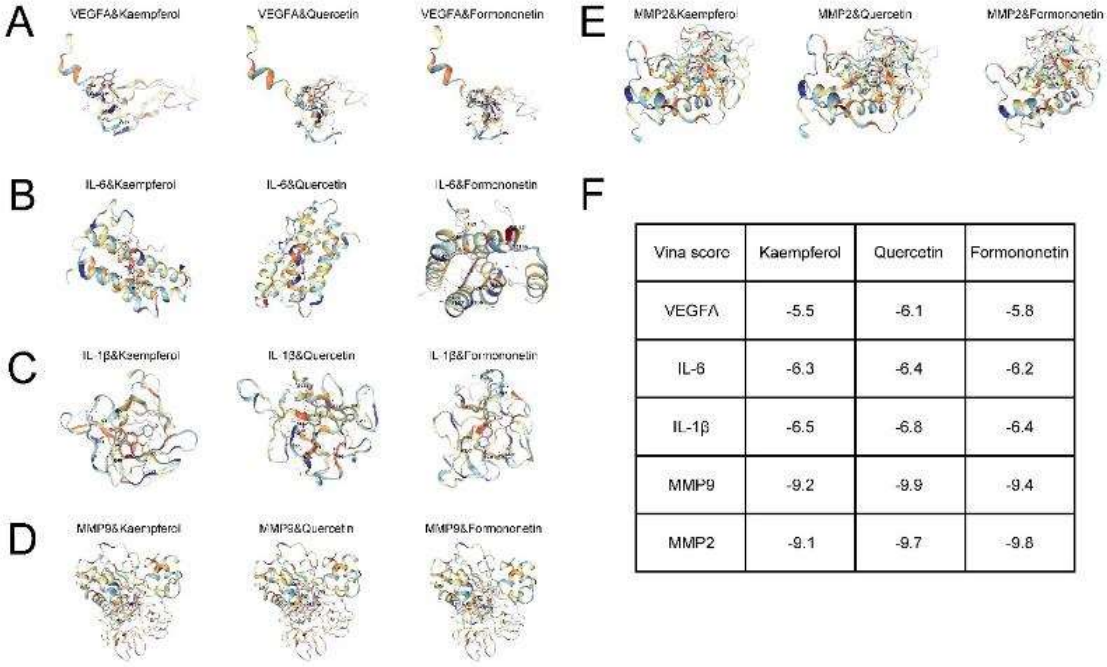



**Figure 3:** PPI network of LDH and *Astragalus membranaceus*. (A) PPI network among the core proteins of *Astragalus membranaceus* intervention for LDH. The edges represent protein-protein and nodes represent proteins; (B) The top 10 targets of *Astragalus membranaceus* in treating LDH were sorted by degree values




| Vina score | Kaempferol | Quercetin | Formononetin |
|---|---|---|---|
| VEGFA | -5.5 | -6.1 | -5.8 |
| IL-6 | -6.3 | -6.4 | -6.2 |
| IL-1β | -6.5 | -6.8 | -6.4 |
| MMP9 | -9.2 | -9.9 | -9.4 |
| MMP2 | -9.1 | -9.7 | -9.8 |

**Figure 4:** Molecular docking of *Astragalus membranaceus* with its key target genes. (A - E) The molecular docking of target genes VEGFA, IL-6, IL-1β, MMP9 and MMP2 with *Astragalus membranaceus* active components containing quercetin, kaempferol and formononetin. (F) The vina score of molecular docking

## DISCUSSION

Lumbar disc herniation affects the lumbar spine and presses on nearby nerves, causing pain, numbness, and weakness in the lower back, buttocks, legs, and feet. In severe cases, cauda equina nerve damage may occur, leading to defecation disorders and even paralysis. Developing complementary and alternative therapies are necessary for LDH treatment [2]. *Astragalus membranaceus* inhibits bone resorption, regulates bone metabolism, and prevents bone loss in patients with primary osteoporosis [3-5]. However, the mechanism of action of *Astragalus membranaceus* in LDH treatment remains unclear.

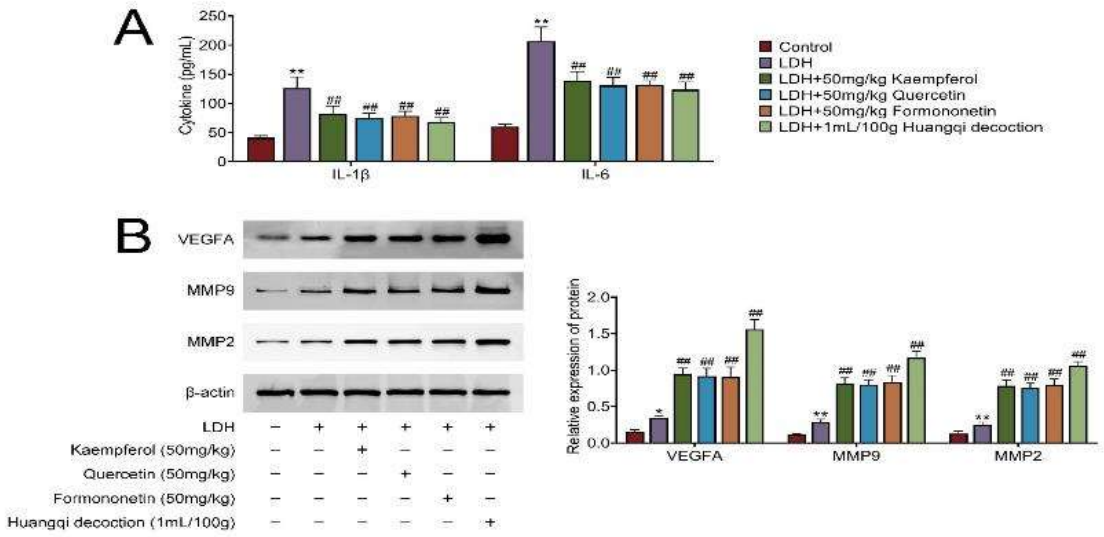



**Figure 5:** Astragalus inhibited the inflammation in LDH model. (A) Concentrations of IL-1β and IL-6 were significantly reduced in groups treated with quercetin, kaempferol, formononetin and Huangqi decoction; (B) The expression levels of VEGFA, MMP-9 and MMP2 were significantly increased when the cells were treated with quercetin, kaempferol, formononetin and Huangqi decoction. Data are presented as mean ± SD. *Compared with control group; #compared with LDH group. *$P<0.05$, **/##$p < 0.01$

In this work, a network pharmacology approach was conducted to analyze *Astragalus membranaceus*. A total of 20 bioactive ingredients of *Astragalus membranaceus* were screened, and 525 targets of these bioactive ingredients were acquired. There were 698 targets of LDH acquired from online databases, and 84 overlapping recognitions between *Astragalus membranaceus*-related targets and LDH-related targets were designated as core targets for further study.

Network pharmacology analysis combines high-throughput histology, bioinformatics, and systems biology [11,12], and which was applied in this study to the therapeutic effects of *Astragalus membranaceus* in LDH treatment [2,13]. The active components of *Astragalus membranaceus* include kaempferol, quercetin and formononetin, which have been widely used clinically for a long time. It is well known that traditional Chinese prescriptions are characterized by multi-components and multi-targets, and cannot be compared to Western drugs which have single chemical components [14]. Direct at the 84 overlapping genes, a new subject of PPI network was conducted for a comprehensive analysis on the illness mechanism of actions and ingredients of *Astragalus membranaceus*.

Molecular docking is used to predict the binding affinity of *Astragalus membranaceus* to its target proteins[7]. This program uses different algorithms and scoring functions to evaluate the binding energy and determine the best conformation of the ligand within the binding site of the target protein. The results of the docking simulations provide insights into the interaction between the ligand and target genes, including the binding site, binding mode, and binding affinity [15]. This information may be instructive for designing new ligands with improved potency and selectivity. Hence, molecular docking is a useful tool for predicting the interaction between *Astragalus membranaceus* and its key target genes.

*Astragalus membranaceus* has been shown to have anti-inflammatory effects in *in vitro* and *in vivo* models [16]. *Astragalus membranaceus* has also been reported to stimulate the production of anti-inflammatory cytokines and inhibit the production of pro-inflammatory cytokines such as IL-1β and IL-6 [17,18]. This may help to reduce the activation of immune cells and the subsequent release of inflammatory mediators. Additional research is required to comprehensively grasp the mechanisms of action and establish the ideal dosage and treatment duration.

## CONCLUSION

This study employed network pharmacology analysis to acquire the active components of *Astragalus membranaceus* and their target genes. GO, KEGG and PPI network analysis demonstrates that VEGFA, AKT1, JUN, PTGS2, IL6, MMP9, IL1β, CXCL8, EGF and MMP2 exert vital activities in LDH treated with *Astragalus*

*membranaceus*. Molecular docking results suggest that these vital proteins bind active components of *Astragalus membranaceus*, and the active components inhibit inflammation in the LDH model. Thus, the data obtained have shed more light on the prevention and treatment of LDH with the traditional Chinese prescription, *Astragalus membranaceus*.

## DECLARATIONS

### *Acknowledgements*

None provided.

### *Funding*

None provided.

### *Ethical approval*

None provided.

### *Availability of data and materials*

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

### *Conflict of Interest*

No conflict of interest associated with this work.

### *Contribution of Authors*

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. WenJie Ke, Chengwei Yu designed the study and carried them out; WenJie Ke, Chengwei Yu, Wei Liu, Haifeng Liu supervised the data collection, analyzed and interpreted the data; WenJie Ke, Chengwei Yu prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript for publication.



## REFERENCES

1. *Amin RM, Andrade NS, Neuman BJ. Lumbar disc herniation. Curr Rev Musculoske 2017; 10: 507-516.*
2. *Gu Y, Zhu H, Wang X, Zhang S, Tong P, Lv S. Exploring the mechanism of Buyang Huanwu decoction in the treatment of lumbar disc herniation based on network pharmacology and molecular docking. Medicine 2022; 101(32): e29534.*
3. *Chai Y, Pu X, Wu Y, Tian X, Li Q, Zeng F, Wang J, Gao J, Gong H, Chen Y. Inhibitory effect of Astragalus Membranaceus on osteoporosis in SAMP6 mice by regulating vitamin D/FGF23/Klotho signaling pathway. Bioengineered 2021; 12(1): 4464-4474.*
4. *Kang S-C, Kim HJ, Kim M-H. Effects of Astragalus membranaceus with supplemental calcium on bone mineral density and bone metabolism in calcium-deficient ovariectomized rats. Biol Trace Elem Res 2013; 151(68-74.*
5. *Park K-R, Park JE, Kim B, Kwon IK, Hong JT, Yun H-M. Calycosin-7-O-β-Glucoside Isolated from Astragalus membranaceus Promotes Osteogenesis and Mineralization in Human Mesenchymal Stem Cells. Int J Mol Sci 2021; 22(21): 11362.*
6. *Huang D, Lv Y, Lu C, Zhang B, Fu Z, Huang Y. Mechanism of Rhizoma Coptidis in epilepsy with network pharmacology. Allergol Immunopath 2022; 50(3): 138-150.*
7. *Guo M-F, Dai Y-J, Gao J-R, Chen P-J. Uncovering the mechanism of Astragalus membranaceus in the treatment of diabetic nephropathy based on network pharmacology. J Diabetes Res 2020; 2020(*
8. *Yi W, Chen Q, Liu C, Li K, Tao B, Tian G, Zhou L, Li X, Shen J, Liu B. LIPUS inhibits inflammation and catabolism through the NF-κB pathway in human degenerative nucleus pulposus cells. J Orthop Surg Res 2021; 16: 1-9.*
9. *Huang J, Dai W, Xiao D, Xiong Q, Liu C, Hu J, Ge F, Yu X, Li S. Acetylation-dependent SAGA complex dimerization promotes nucleosome acetylation and gene transcription. Nat Struct Mol Biol 2022; 29(3): 261-273.*
10. *Mukherjee A, Park A, Davies KP. PROL1 is essential for xenograft tumor development in mice injected with the human prostate cancer cell-line, LNCaP, and modulates cell migration and invasion. J Mens Health 2022; 18(2).*
11. *Yang J, Zhang X, Jiang Z, Deng Z, Tang J. Analysis of the drug-target-disease network of trichosanthes-angelica sinensis-frankincense-myrrh in the treatment of breast cancer. Eur J Gynaecol Oncol 2022; 43(6): 83-95.*
12. *Ma Z, Zhao P. A network pharmacology-based investigation of the mechanism involved in the anti-gastric cancer effect of Oldenlandia diffusan, a traditional Chinese medicine. Trop J Pharm Res 2022; 21(11): 2403-2410.*

13. Sun K, Zhu L-G, Wei X, Zhang P, Zhan J-W, Wang Y, Yin H. Study on mechanism of" Epimedii Folium-Paeoniae Radix Alba" in treatment of lumbar disc herniation based on network pharmacology. Zhongguo Zhong yao za zhi 2020; 45(3): 609-616.
14. Fan Z, Chen J, Yang Q, He J. Network Pharmacology and experimental validation to reveal the pharmacological mechanisms of chongcaoyishen decoction against chronic kidney disease. Front Mol Biosci 2022; 9.
15. Jiao X, Jin X, Ma Y, Yang Y, Li J, Liang L, Liu R, Li Z. A comprehensive application: Molecular docking and network pharmacology for the prediction of bioactive constituents and elucidation of mechanisms of action in component-based Chinese medicine. Comput Biol Chem 2021; 90(107402.
16. Chen G, Jiang N, Zheng J, Hu H, Yang H, Lin A, Hu B, Liu H. Structural characterization and anti-inflammatory activity of polysaccharides from Astragalus membranaceus. Int J Biol Macromol 2023: 124386.
17. Huang WY, Pan JH, Jeong I, Oh SJ, Hyun YG, Kim MS, Han BK, Hong J, Koo YT, Lee KW. Antifatigue and Anti-Inflammatory Effects of Cervus elaphus L., Angelica gigas Nakai, and Astragalus membranaceus Bunge Complex Extracts in Physically Fatigued Mice. J Med Food 2022; 25(12): 1126-1132.
18. Lai PKK, Chan JYW, Cheng L, Lau CP, Han SQB, Leung PC, Fung KP, Lau CBS. Isolation of anti-inflammatory fractions and compounds from the root of Astragalus membranaceus. Phytother Res 2013; 27(4): 581-587.