ISSN 2616-5791 Vol. 6, Issue 7: 46-52, DOI: 10.25236/AJMHS.2025.060707

Network Pharmacology-Based Exploration of Multi-Target Synergistic Mechanisms of Tripterygium Wilfordii and Valsartan in Diabetic Nephropathy

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Abstract: *This study employed network pharmacology to investigate the synergistic mechanisms of Tripterygium wilfordii (TW) and valsartan for diabetic nephropathy (DN). Network pharmacology analysis identified 67 overlapping core targets. A PPI network (67 nodes, 171 edges) prioritized key hub genes (ACE, AGT, AKT1, BCL2, CASP3, CCL2, CREB1, CXCL8, ESR1, JUN). Gene Ontology enrichment (p-value<0.001, FDR<0.05) revealed significant involvement in inflammatory response, blood pressure regulation, hypoxia response, and apoptosis. KEGG pathway analysis (p<0.001) highlighted crucial signaling pathways, including HIF-1, TNF, IL-17, MAPK, and PI3K-Akt. These results demonstrate that TW and valsartan likely exert combined therapeutic effects on DN through multi-target, multi-pathway modulation, providing a theoretical basis for optimized combination therapy and guiding future experimental validation.*

Keywords: *Tripterygium Wilfordii, Traditional Chinese Medicine, Diabetic Nephropathy, Valsartan, Immune Regulation, Network Pharmacology*

1. Introduction

Diabetic Nephropathy (DN) is one of the common and serious microvascular complications of diabetes. Its primary clinical features are persistent albuminuria and progressive decline in glomerular filtration rate, which can ultimately advance to End-Stage Renal Disease (ESRD). Globally, DN is the leading cause of ESRD, accounting for over 40% of cases [1]. In China, the prevalence of diabetes among adults is approximately 10.9%, with the prevalence of DN among these patients reaching as high as 33.6% [2]. Proteinuria is an independent risk factor for the progression of DN. Its occurrence is associated with multiple mechanisms, among which podocyte injury is the most critical [3, 4]. Podocytes are the core cells responsible for maintaining the structure and function of the glomerular filtration barrier. Under the influence of multiple factors such as hyperglycemia, accumulation of advanced glycation end products (AGEs), oxidative stress, inflammatory responses, TGF-β1 activity, activation of the renin-angiotensin system (RAS), and mechanical stress, podocytes undergo injury. This leads to damage of the glomerular filtration barrier, resulting in substantial proteinuria [5]. Therefore, reducing proteinuria is crucial for slowing the progression of DN. However, a significant proportion of patients still ultimately require dialysis treatment due to uncontrolled proteinuria.

Current Western medical treatment for DN primarily employs angiotensin converting enzyme inhibitors (ACEI) / angiotensin receptor blockers (ARB) drugs, with therapeutic goals including blood glucose control, blood pressure management, lipid regulation, and anticoagulation. However, the key mechanisms driving DN progression involve the activation of the RAS and inflammatory responses [4]. Clinical studies have confirmed that RAS blockers (i.e., ACEI/ARB drugs) provide renoprotective effects independent of their blood pressure-lowering actions. For example, the angiotensin II receptor antagonist valsartan inhibits RAS activity by blocking the binding of angiotensin II to its receptor. Its renoprotective mechanisms include ameliorating the states of glomerular hyperfiltration, hypertension, and hyperperfusion, thereby delaying glomerulosclerosis, preserving renal function, and reducing proteinuria. Nonetheless, in DN patients with massive proteinuria, the therapeutic efficacy of valsartan is often limited in DN patients with massive proteinuria [6, 7].

Extracts of the traditional Chinese herb Tripterygium wilfordii (TW) contain multiple active

ISSN 2616-5791 Vol. 6, Issue 7: 46-52, DOI: 10.25236/AJMHS.2025.060707

components, such as triptolide, tripdiolide, and triptonide, which typically exert synergistic pharmacological effects [8]. TW possesses traditional properties including dispelling wind-dampness, clearing heat-toxicity, reducing swelling and dissipating nodules, and activating blood circulation to resolve stasis [9-11]. Modern pharmacological studies indicate that TW extracts exhibit effects such as anti-inflammation, anti-oxidation, and immunosuppression, and can inhibit the proliferation of glomerular mesangial cells and matrix. Concurrently, it can reduce anionic charge loss in the glomerular basement membrane, stabilize and repair the podocyte cytoskeleton, thereby protecting the glomerular filtration barrier and reducing proteinuria [12, 13].

Building on this foundation, this study employed network pharmacology to systematically analyze potential interactions following the combined use of TW and Valsartan. The research aims to predict potential targets and pathways of drug interactions between these two agents, thereby providing clues for designing focused subsequent experiments and optimizing the allocation of research resources. Simultaneously, the findings are expected to offer a theoretical basis for rational clinical combination therapy, enhancing therapeutic efficacy, and reducing adverse reactions.

2. Methods

2.1 Collection of Potential TW Constituents and Targets

Potential TW constituents were collected using the Traditional Chinese Medicine Systematic Pharmacology Database and Analysis Platform (TCMSP), with oral bioavailability (OB) ≥ 30%, molecular weight(MW) < 500, Half-life (HL) > 4, and drug-like properties (DL) ≥ 0.18 as the screening conditions for the constituents, supplemented by literature search. Their chemical structures and properties, such as MW, BBB, HL, OB, and DL, were retrieved from TCMSP. Finally, Pubchem and Swiss Target Prediction were used to collect the targets of compounds, set the species to Homo sapiens, and establish their target database.

2.2 Collection of Potential Valsartan Targets

Targets related to valsartan were searched using "valsartan" keywords in the GeneCards database. The screening condition was set to relevance > 1 and Category = Protein Coding.

2.3 Diabetic Nephropathy Target Gene Screening

Targets related to Diabetic nephropathy were searched using "Diabetic nephropathy" keywords in the GeneCards database. The screening condition was set to relevance > 2 and Category = Protein Coding.

2.4 Obtaining the Core Targets of TW and Valsartan Acting on Diabetic Nephropathy

This study combined the collected targets of TW with those of valsartan to create a synergistic target library. Then, we identified the core targets of this combination acting on diabetic nephropathy and generated a Venn diagram using Venny, an online plotting tool (https://bioinfogp. cnb.csic. es/tools/venny/).

2.5 Protein Interaction Analysis of Overlapping Genes

The core targets were entered into the STRING website to construct the protein-protein interaction (PPI) network. The species "Homo sapiens" was selected on the website, with highest confidence (0.900), while free proteins were hidden to obtain the PPI interaction network relationship map.

2.6 Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Enrichments

Furthermore, to analyze the TW-valsartan combination's core targets for Diabetic Nephropathy within biological processes (BP), molecular functions (MF), cellular components (CC), and KEGG pathways, the collected gene data were imported into the DAVID database with 'Homo sapiens' set as the species. GO and KEGG pathway enrichment analyses were performed for the core targets. The top 10 GO terms and KEGG pathways were selected and imported into the Microbiology Letter website (www.bioinformatics.com.cn) to obtain GO histograms and KEGG enrichment maps.

ISSN 2616-5791 Vol. 6, Issue 7: 46-52, DOI: 10.25236/AJMHS.2025.060707

3. Results

3.1 Main TW Constituents and Their Targets

Using OB ≥ 30%, MW < 500, HL > 4, and DL ≥ 0.18 as screening conditions, 16 TW chemical constituents were retrieved from TCMSP. The compounds and their biochemical properties are summarized in Table 1, where OB is the oral bioavailability, and DL refers to the similarity of a compound to a known drug, an important indicator to evaluate whether a compound can be used as a drug or not. Finally, 135 targets of these active ingredients were collected by the Swiss Target Prediction.

Table 1: Information of potential constituents of TW.

Name	MV	OB(100%)	DL	HL
hederagenin	414.79	36.91	0.75	5.35
81827-74-9	342.47	45.42	0.53	5.58
triptolide	360.44	51.29	0.68	4.14
Tryptophenolide	312.44	48.5	0.44	4.42
Celallocinnine	405.59	83.47	0.59	10
Isoxanthohumol	354.43	56.81	0.39	17.98
Tripdiotolnide	360.44	56.4	0.67	4.91
21-Hydroxy-30-norhopan-22-one	428.77	34.11	0.77	6.66
TRIPTONOLIDE	326.42	49.51	0.49	17.94
beta-sitosterol	414.79	36.91	0.75	5.36
Mairin	456.78	55.38	0.78	8.87
kaempferol	286.25	41.88	0.24	14.74
Stigmasterol	412.77	43.83	0.76	5.57
nobiletin	402.43	61.67	0.52	16.2
[(2S)-2-[[(2S)-2-(benzoylamino)-3-phenylpropanoyl]amino]-3-phenylpropyl] acetate	444.57	58.02	0.52	6.03
(5S,8S,9S,10R,13R,14S,17R)-17-[(1R,4R)-4-ethyl-1,5-dimethylhexyl]-10,13-dimethyl-2,4,5,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,6-dione	428.77	33.12	0.79	6.56

3.2 The Core Targets of TW and Valsartan Acting on Diabetic Nephropathy

Initial database screening using GeneCards identified 82 valsartan-related targets and 417 Diabetic Nephropathy-associated targets through keyword searches ("Valsartan" and "Diabetic Nephropathy"). Following intersection of the TW and valsartan targets, 201 synergistic targets were identified. Ultimately, Venn analysis revealed 67 overlapping core targets (Figure 1, Table 2), indicating shared therapeutic mechanisms of TW and valsartan against diabetic nephropathy.

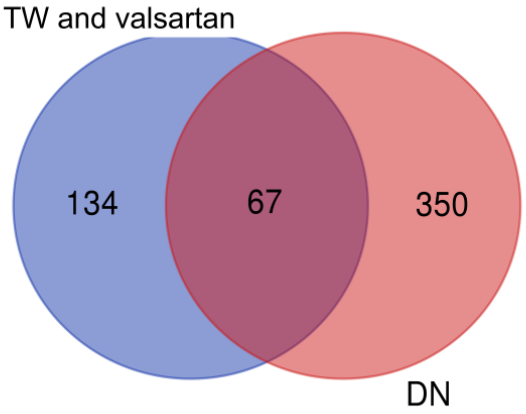

Figure 1: Overlapping core targets of TW and valsartan against Diabetic Nephropathy.

ISSN 2616-5791 Vol. 6, Issue 7: 46-52, DOI: 10.25236/AJMHS.2025.060707

Table 2: The overlapping core targets.

ACE	NPPB	MAPK3	SELE	AKT1	PTX3	CCL2
HMGCR	SOD1	APOB	NPPA	STAT3	ADRB2	PRKCA
EDN1	CD79A	AKR1B1	SERPINE1	PTGS2	VEGFA	STAT1
KDR	CREB1	AGT	MAPK14	AGER	TGFB1	PRSS1
HMOX1	PON1	PPARG	VCAM1	SIRT1	KCNJ11	CASP3
NOS2	IFNG	CRP	TP53	REN	MME	MMP9
INS	IL4	RELA	ADRB1	ESR1	JUN	ADIPOQ
MAPK1	BCL2	NOS3	TNF	AGTR1	TIMP1	
PIK3CG	MAPK8	CXCL8	LPL	ALB	AGTR2	
DPP4	ICAM1	KNG1	SELP	APOA1	EPO	

3.3 The PPI network analysis

The overlapping core targets were analyzed using the STRING database to construct a protein-protein interaction (PPI) network (Figure 2). This network comprised 67 nodes and 171 edges, with multiple interaction relationships observed between protein pairs. Hub genes were prioritized by degree of connectivity, with the following top-ranked targets identified: ACE, AGT, AKT1, BCL2, CASP3, CCL2, CREB1, CXCL8, ESR1, and JUN. Higher degree centrality values indicate greater topological importance within the network, suggesting these genes function as key regulatory elements in the therapeutic mechanism.

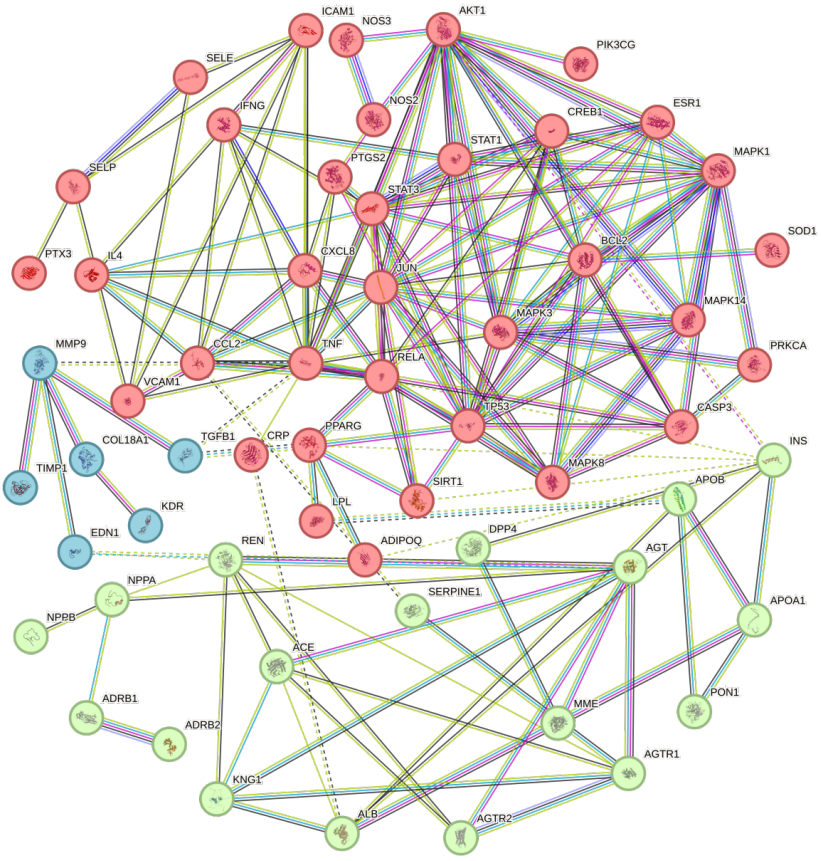

Figure 2: PPI network screening of the overlapping core targets.

3.4 GO enrichment

Gene Ontology (GO) enrichment analysis of the overlapping core targets was conducted using the DAVID database with significance thresholds set at p-value < 0.001 and FDR < 0.05. This analysis identified 146 biological process (BP) terms, 16 cellular component (CC) terms, and 15 molecular function (MF) terms. The top 10 significantly enriched terms from each GO category were visualized using bar charts, respectively (Figure 3).

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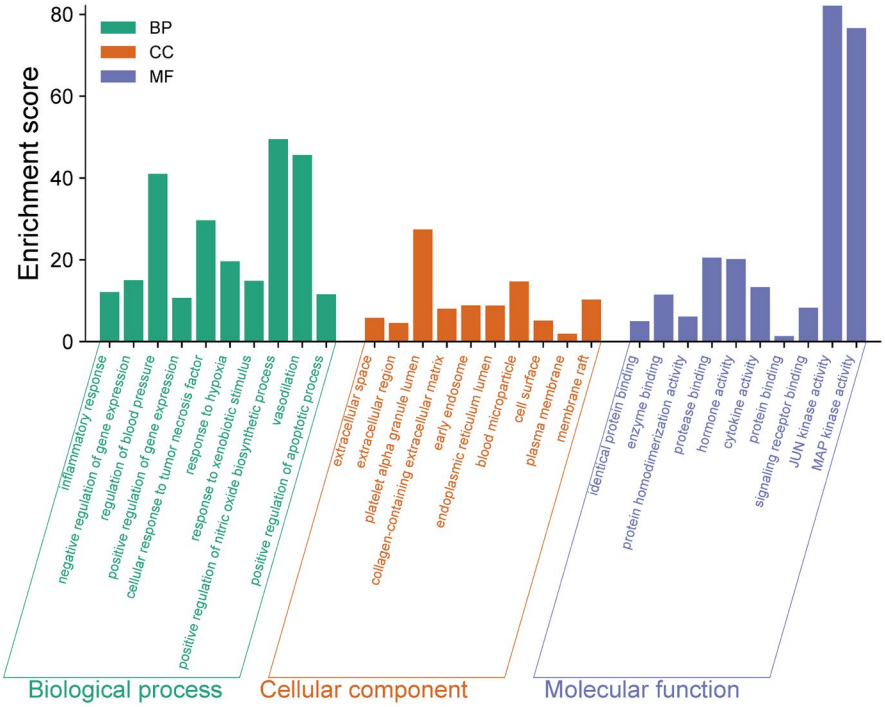

Figure 3: GO enrichment of the overlapping core targets

3.5 KEGG pathway enrichment

KEGG pathway enrichment analysis of the overlapping core targets was performed using the DAVID database with a significance threshold of p-value < 0.001. This analysis identified 106 significantly enriched pathways. The top 15 pathways were visualized using bubble plots (Figure 4). These results demonstrate that TW and valsartan likely exert therapeutic effects on diabetic nephropathy through coordinated modulation of multiple signaling pathways.

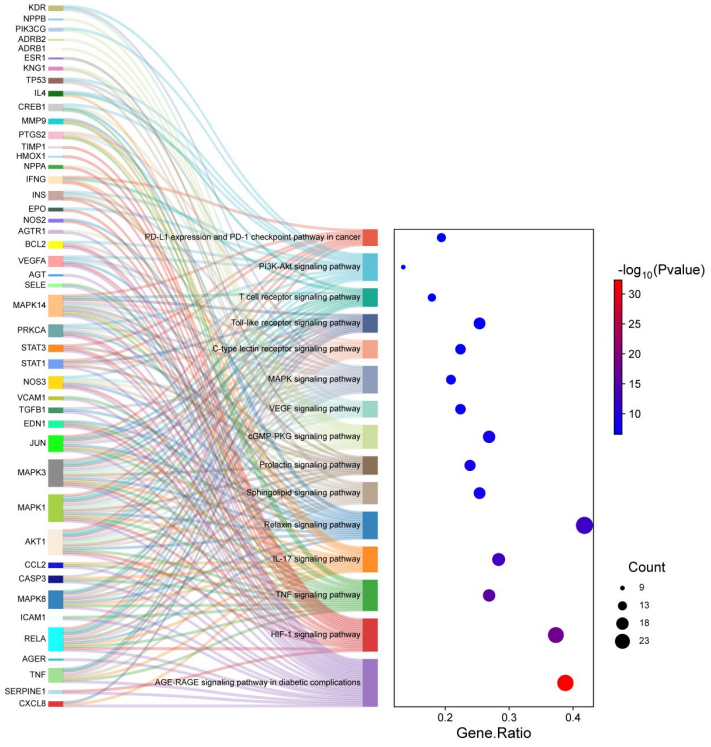

Figure 4: KEGG enrichment of the overlapping core targets

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4. Discussion

Network pharmacology, systematically proposed by Andrew L. Hopkins, integrates systems biology, bioinformatics, and polypharmacology to elucidate the complex "disease-gene-target-drug" interaction networks. As an efficient and economical method, it facilitates rapid target screening and mechanistic exploration of drug actions [14, 15]. Its core characteristics—holism, a systems approach, and focus on drug interactions—align closely with Traditional Chinese Medicine's (TCM) holistic philosophy and compound compatibility theory, making it particularly well-suited for studying the complex mechanisms of Chinese herbal medicines [16-18].

Integrating network pharmacology and bioinformatics, we reveal the synergistic mechanisms of TW-valsartan co-therapy for diabetic nephropathy. The identification of 67 overlapping core targets and the construction of a PPI network highlighted central hub genes like ACE, AGT, AKT1, BCL2, CASP3, CCL2, CREB1, CXCL8, ESR1, and JUN. Their high degree centrality strongly suggests these are critical regulatory nodes mediating the combined effects. This aligns with the known roles of these targets in DN pathophysiology, including inflammation (CCL2, CXCL8), apoptosis (BCL2, CASP3), fibrosis (AGT), and vascular function (ACE) [19].

Clinical research demonstrates that major TW preparations (e.g., Tripterygium glycosides) combined with RAS blockers can synergistically target RAS activation and inflammatory responses in patients with DN. Meta-analysis results further confirm that Tripterygium glycosides combined with valsartan can significantly improve clinical response rates, reduce urinary protein excretion, and elevate serum albumin levels; meanwhile, relevant renal function indicators (such as serum creatinine, blood urea nitrogen, and endogenous creatinine clearance rate) remain stable. Owing to these advantages, this combination regimen has become a research hotspot in integrated Chinese-Western medicine for DN treatment [20, 21].

The extensive enrichment analyses further solidified the predicted mechanisms. The significant GO terms, particularly in Biological Processes (e.g., inflammatory response, regulation of blood pressure, response to hypoxia, positive regulation of apoptosis), and Molecular Functions (e.g., cytokine activity, kinase activity), point towards a multi-faceted action targeting inflammation, hemodynamics, cellular stress responses, and programmed cell death - all hallmarks of DN progression [22]. Crucially, the KEGG pathway analysis revealed a highly coordinated modulation of critical signaling cascades. The enrichment of pathways like HIF-1 signaling, TNF signaling, IL-17 signaling, MAPK signaling, and the PI3K-Akt signaling pathway strongly supports the hypothesis that the TW-valsartan combination likely exerts its therapeutic benefit by simultaneously dampening inflammation, mitigating hypoxia-induced damage, regulating cell survival/proliferation, and improving vascular function [23]. The inclusion of pathways like the PD-L1/PD-1 checkpoint in cancer also intriguingly hints at potential immunomodulatory effects relevant to DN's inflammatory component.

5. Conclusion

In conclusion, the findings offer a compelling theoretical rationale for the clinical co-administration of TW and valsartan, suggesting potential for enhanced efficacy (through multi-pathway synergy) and possibly reduced adverse effects (through lower individual dosing requirements), warranting careful clinical investigation.

Acknowledgements

This study was supported by the Sichuan Hospital Association's 2022 Young Pharmacists Research Project (YP2202424), and 2025 Nanchong City Social Science Planning Project - Special Research Project on Health Humanities in Northern Sichuan(NC25CB13, NC25CB54)

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