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Initial Investigation into the Therapeutic Effects and Pharmacological Mechanisms of Modified Danggui-Shaoyao San for Managing Depression in Chronic Kidney Disease Patients

Isabel Romero[1*], Marta Campos[1]

[1]Department of Phytochemistry, Faculty of Pharmacy, Complutense University of Madrid, Madrid, Spain.

***E-mail** ✉ isabel.romero.es@gmail.com

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ABSTRACT

Depression is a common complication in Chronic Kidney Disease (CKD) that significantly impacts patient prognosis. Modified Danggui-Shaoyao-San (MDSS), an optimized version of the traditional Chinese formula Danggui-Shaoyao-San (DSS), has been developed for depression management. This study aimed to assess the clinical efficacy and safety of MDSS in treating depression in CKD patients and to explore its underlying molecular mechanisms using pharmacological analysis and molecular docking. A total of 62 patients were randomly assigned to a treatment group receiving MDSS or a control group receiving placebo. Depression severity was evaluated using the Hamilton Depression Scale, with the primary outcomes being improvement in depressive symptoms and effects on liver and kidney function and electrolyte balance. Core compounds and potential targets of MDSS were identified through the TCMSP database, while CKD- and depression-related molecular targets were obtained from GeneCards, OMIM, and DisGeNET databases. A protein–protein interaction (PPI) network was constructed using the STRING database, and key targets were analyzed via Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment. Molecular docking was performed to validate the interactions between major active compounds and target proteins. Clinically, patients treated with MDSS showed significant improvement in depressive symptoms without notable adverse effects. Network pharmacology revealed a compound-target network comprising 47 compounds and 69 corresponding targets. GO enrichment analysis identified 844 significant terms, and KEGG pathway analysis highlighted 254 relevant signaling pathways. Molecular docking confirmed strong binding affinities between the top active compounds and four key target proteins. This study provides preliminary evidence supporting the effectiveness of MDSS in alleviating depression in CKD patients and highlights its multi-compound, multi-target pharmacological characteristics.

Keywords: Network pharmacology, Depression, Modified Danggui-Shaoyao San, Clinical efficacy, Chronic kidney disease

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Introduction

Depression is a major contributor to the global disease burden [1] and occurs frequently as a comorbidity in patients with chronic kidney disease (CKD), with prevalence rates up to five times higher than in the general population [2]. Depression in CKD not only worsens clinical symptoms but also reduces patients' quality of life and adherence to treatment [3], while substantially increasing the risk of cardiovascular events and mortality [4]. Current standard treatment for depression in CKD primarily involves Western pharmacotherapy, which is often associated with significant hepatic and renal toxicity [5]. Renal impairment in CKD patients exacerbates these adverse effects, and concerns about potential renal damage often lead clinicians to reduce antidepressant dosages or avoid prescribing them altogether, limiting treatment options for depression in this population [6].

Danggui-Shaoyao-San (DSS), a traditional Chinese medicinal formula documented in the classical text Synopsis of Golden Chamber, mainly comprises Angelica and Paeoniae and has historically been used to nourish the heart and calm the mind. Modern research supports its efficacy in alleviating depressive symptoms [7, 8]. Recently, renowned traditional Chinese medicine practitioners in Sichuan have modified DSS by incorporating kidney- and diuretic-nourishing herbs such as Rehmanniae, Poria, and Amomum, which support liver and kidney function, forming Modified Danggui-Shaoyao-San (MDSS). In MDSS, Rehmanniae targets the kidneys and promotes diuresis, whereas Angelica supports liver yin, nourishes blood, and improves circulation, making MDSS a widely used formula for managing depression in CKD. However, robust clinical evidence regarding the efficacy and pharmacological mechanisms of MDSS remains lacking.

This study aimed to address this gap by evaluating the clinical efficacy and safety of MDSS in treating depression in CKD patients through a 6-week single-blind randomized clinical trial. Additionally, network pharmacology combined with molecular docking was employed to explore the molecular mechanisms underlying MDSS's therapeutic effects, providing essential data for future basic research and clinical application.

Materials and Methods

Clinical efficacy study

A randomized, controlled, single-blind study design was employed. The study protocol was approved by the Second Clinical Medical Institution of North Sichuan Medical College (Grant: 2020–134) and conducted in accordance with the Declaration of Helsinki and its amendments. The trial was registered in the Chinese Clinical Trial Registry (www.chictr.org.cn; registration number: ChiCTR2100041867). Written informed consent was obtained from all participants or their legal representatives prior to enrollment. Participants were informed that they could withdraw from the study at any time without affecting their medical care or relationship with the investigators.

Inclusion and exclusion criteria

Eligible participants met the DSM-5 diagnostic criteria for depression [9] and CKD stages 1–3 according to KDIGO criteria [10]. Traditional Chinese Medicine (TCM) diagnostic criteria for kidney deficiency and liver depression were applied based on the Guidelines for the Treatment of Common Diseases in Internal Medicine [11]. Primary symptoms included depressed mood, anhedonia, irritability, slowed thinking, fatigue, insomnia, memory impairment, loss of libido, and decreased appetite, while secondary symptoms comprised discouragement, lethargy, soreness and weakness of the waist and knees, chest distension, tightness, shortness of breath, pale or dull complexion, tongue abnormalities, and a sunken wiry pulse.

Inclusion criteria were: i) meeting the above TCM and Western diagnostic criteria; ii) aged 18–65 years; iii) Hamilton Depression Scale (HAM-D) score ≥7; iv) informed consent provided. Exclusion criteria included: i) unstable vital signs; ii) CKD stage 5D dialysis or CKD stage 3–4 with oliguria/anuria; iii) severe aphasia or cognitive impairment preventing communication; iv) schizophrenia; v) depression secondary to other psychiatric disorders (e.g., bipolar disorder, OCD, schizophrenia); vi) anxiety disorders linked to physical disease; vii) known alcohol or substance dependence. Patients meeting any exclusion criteria were not enrolled.

Intervention

A total of 62 patients admitted between December 2020 and December 2021 at Santai County Traditional Chinese Medicine Hospital were randomized into treatment (n=31) and control (n=31) groups using a random number table. The treatment group received MDSS (15g Rehmanniae, 15g Angelicae, 15g Paeoniae, 20g Rhizoma, 15g Atractylodes, 15g Poria, 12g Alisma, 10g Amomum, 15g Hordei Fructus Germinatus) prepared as 8 doses of 20 g/day, administered in the early morning for 6 weeks. The control group received a placebo at the same time points for the same duration.

Outcome measures

The primary outcome was change in HAM-D scores at baseline, and weeks 2, 4, and 6. HAM-D is widely used to assess depression severity, with higher scores indicating more severe depression [12]. Secondary outcomes included: i) partial responders (25–50% HAM-D reduction), ii) responders (≥50% reduction), iii) remitters (HAM-D ≤7), and iv) comparison of response and remission rates between groups [13]. Additionally, blood creatinine

(CR), blood urea nitrogen (BUN), serum alanine aminotransferase (ALT), glutamate aminotransferase (AST), and potassium (K+) levels were measured at baseline and after 6 weeks to evaluate effects on liver and kidney function and electrolytes, while adverse reactions were monitored.

Statistical analysis

Continuous variables were presented as mean ± standard deviation (SD), and categorical variables as frequencies and percentages. Categorical data were compared using chi-square or Fisher's exact test, as appropriate. Paired t-tests assessed within-group changes before and after intervention, while independent t-tests compared between-group means. A p-value <0.05 was considered statistically significant. Analyses were performed using SPSS version 19 (IBM, USA).

Collection of MDSS component targets

The herbal ingredients of MDSS, including Rehmanniae, Angelicae, Paeoniae, Rhizoma, Atractylodes, Poria, Alisma, Amomum, and Hordei Fructus Germinatus, were primarily gathered from the TCMSP database (http://tcmspw.com/tcmsp.php) [14]. Compounds with oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 were selected, and their associated protein targets were identified [15]. These protein targets were subsequently mapped to their corresponding human genes using the UniProt database (https://www.uniprot.org/) [16].

Identification of depression-associated targets in CKD

To obtain genes relevant to depression in CKD, searches were conducted in GeneCards (https://www.genecards.org/) [17], OMIM (https://omim.org/) [18], and DisGeNET (https://www.disgenet.org/home/) [19] using the keywords "CKD" and "Depression." Redundant entries were removed to generate a refined list of candidate genes. Overlapping targets between the MDSS-related genes and CKD-depression-associated genes were determined using a Venn diagram, representing potential molecular targets through which MDSS may exert therapeutic effects.

Construction of compound–target and protein–protein interaction (PPI) networks

The identified bioactive compounds and overlapping genes were imported into Cytoscape 3.8.2 to construct a compound-target network for MDSS in depression associated with CKD. Intersected genes were also analyzed using the STRING database (https://string-db.org/) [20], specifying the species as Homo sapiens and removing isolated nodes, to generate a PPI network, which was visualized and further analyzed in Cytoscape.

Functional enrichment and pathway analysis

Gene Ontology (GO) enrichment for biological processes and KEGG pathway analyses were conducted using the Metascape platform (http://metascapape.org) [21]. The intersected gene list was submitted with official gene symbols, with the species restricted to Homo sapiens. Significant biological processes and pathways were identified using a cutoff of $P < 0.05$.

Molecular docking studies

Proteins with the highest connectivity in the PPI network were selected as key targets for docking with active compounds. UniProt IDs for these proteins were retrieved from the UniProt database. Their 3D structures were downloaded from the RCSB PDB (http://www.rcsb.org/) [22] and prepared by removing water molecules and ligands using PyMOL 2.4.0. 3D structures of active compounds were obtained from PubChem (http://pubchem.ncbi.nlm.nih.gov/) [23]. Docking was carried out with AutoDock Vina 1.1.2 following active pocket definition in AutoDockTools 1.5.6. 3D docking results were visualized using PyMOL 2.4.0, and 2D interaction diagrams were generated using Proteins Plus (https://proteins.plus/).

Patient demographics

A total of 62 participants were randomized equally into the treatment and placebo groups. The treatment group included 14 males and 17 females with an average age of 47.0 ± 10.5 years, whereas the placebo group included 16 males and 15 females with an average age of 48.3 ± 10.5 years. Baseline comparisons of gender, age, HAM-

D scores, and biochemical parameters revealed no statistically significant differences between the two groups **(Table 1).**

Table 1. Baseline Characteristics of the Patients

	Treatment Group (n=31)	Control Group (n=31)	*p*-value
Age (Years), mean (SD)	47.0(10.5)	48.3(10.5)	0.64
Sex, n (%)			0.61
Male	14(45.2)	16(51.6)	
Female	17(54.8)	15(48.4)	
Baseline, mean (SD)			
HAM-D score,	14.8(4.5)	13.6(3.6)	0.23
CR (μmol/L)	207.5(99.2)	202.1(93.2)	0.83
BUN (mg/dl)	43.8(20.0)	45.4(23.6)	0.77
AST (U/L)	20.8(10.2)	21.8(9.5)	0.70
ALT(U/L)	30.4(14.5)	31.2(12.5)	0.81
K^+ (mmol/L)	4.3(0.4)	4.2(0.5)	0.32

Note: P-value of < 0.05 was considered statistically significant.
Abbreviation: SD, Standard deviation.

Clinical outcomes and safety

At baseline, there was no significant difference in HAM-D scores between the treatment group and the control group (14.83 ± 4.48 vs. 13.58 ± 3.47, p = 0.227). After 6 weeks of intervention, the difference in HAM-D scores between the two groups remained statistically non-significant (p = 0.114). However, within the treatment group, HAM-D scores showed a significant reduction from baseline starting at week 2 (p = 0.001), and this improvement persisted through the 6-week study period. In contrast, the control group exhibited no significant change in HAM-D scores compared with baseline.

Specifically, from baseline to week 2, HAM-D scores decreased by 1.16 ± 1.75 in the treatment group versus 0.00 ± 2.03 in the control group, with a significant between-group difference (p = 0.019). By week 4, the reductions were 2.39 ± 2.74 in the treatment group and -0.03 ± 3.31 in the control group (p = 0.003). At week 6, the HAM-D score changes were 3.39 ± 3.29 in the treatment group and 0.16 ± 4.53 in the control group, again showing a significant difference (p = 0.002) **(Table 2).**

Analysis of response categories further supported the efficacy of MDSS. The proportions of patients achieving partial response (25–50% reduction in HAM-D) were 35.5% in the treatment group and 25.8% in the control group. Complete responders (≥50% reduction) accounted for 3.2% in the treatment group and 0% in the control group. Remission, defined as HAM-D ≤7, was observed in 22.6% of the treatment group compared with 12.9% of the control group **(Table 3).**

Table 2. Comparison of HAM-D Score of the Included Individuals

HAM-D Score	Treatment Group	*P1*-value	Control Group	*P2*- value	*P3*- value
Pre-treatment	14.83(4.48)		13.58(3.57)		0.227
Post-treatment 2 weeks	13.67(4.45)	**0.001**	13.58(4.13)	1.00	0.930
Post-treatment 4 weeks	12.45(4.53)	**< 0.001**	13.61(4.56)	0.96	0.319
Post-treatment 6 weeks	11.45(4.37)	**< 0.001**	13.42(5.25)	0.84	0.114
Change in 2 weeks	1.16(1.75)		0.00(2.03)		**0.019**
Change in 4 weeks	2.39(2.74)		−0.03(3.31)		**0.003**
Change in 6 weeks	3.39(3.29)		0.16(4.53)		**0.002**

Notes: Values are presented as mean ± standard deviation (SD). P_1 denotes the paired t-test comparing pre- and post-intervention data within the treatment group; P_2 denotes the paired t-test comparing pre- and post-intervention data within the control group; P_3 represents the independent-samples t-test comparing outcomes between the treatment and control groups after intervention. A p-value <0.05 was considered statistically significant and is highlighted in bold.

Table 3. Comparison the Response to Treatment and Remission Rates in Two Groups

HAM-D Score		Treatment Group	Control Group	P-value
Partial responders, n (%)	2 weeks	1(3.2)	0(0)	1.00
	4 weeks	9(29.0)	2(6.5)	0.46
	6 weeks	11(35.5)	8(25.8)	0.41
Responders, n (%)	2 weeks	0(0)	0(0)	--
	4 weeks	0(0)	0(0)	--
	6 weeks	1(3.2)	0(0)	1.00
Remissions, n (%)	2 weeks	0(0)	3(9.7)	0.24
	4 weeks	2(6.5)	3(9.7)	1.00
	6 weeks	7(22.6)	4(12.9)	0.51

Notes: Data are expressed as mean ± SD. A p-value <0.05 was considered statistically significant.

No significant differences were observed in BUN, creatinine (CR), AST, ALT, or K^+ levels between the treatment and control groups after the intervention, nor were there significant changes within either group when comparing pre- and post-intervention values **(Table 4)**. These results suggest that MDSS administration did not cause further impairment of renal function or adverse effects such as liver dysfunction or hyperkalemia. During the trial, 2 patients in the treatment group reported constipation and 1 patient experienced sweating, while 2 patients in the control group reported headaches. No serious adverse events or deaths occurred in either group.

Table 4. Comparison the Biochemical Data of the Included Individuals

Biochemical Data/Domains	Treatment Group		*P1*-value	Control Group		*P2*-value	*P3*-value
	Pre-	Post-		Pre-	Post-		
CR (umol/L)	207.5(99.2)	109.5(88.2)	0.052	202.1(93.2)	207.1(83.2)	0.549	0.568
BUN (mg/dl)	43.8(20.0)	43.5(20.8)	0.200	45.4(23.6)	46.0(21.8)	0.838	0.647
AST (U/L)	20.8(10.2)	22.1(8.9)	0.591	21.8(9.5)	23.0(7.8)	0.434	0.694
ALT(U/L)	30.4(14.5)	28.2(11.7)	0.492	31.2(12.5)	33.5(11.9)	0.422	0.078
K+(mmol/L)	4.3(0.4)	4.4(0.6)	0.598	4.2(0.5)	4.2(0.5)	0.782	0.247

Notes: Data are presented as mean ± SD. P_1 indicates a paired t-test comparing pre- and post-intervention values within the treatment group; P_2 indicates a paired t-test comparing pre- and post-intervention values within the control group; P_3 represents an independent-samples t-test comparing outcomes between the treatment and control groups after intervention. A p-value <0.05 was considered statistically significant.

Screening of bioactive compounds and corresponding targets

A total of 47 bioactive compounds were identified from the nine herbs in MDSS, meeting the criteria of OB ≥30% and DL ≥0.05. These included 2 compounds from Angelicae, 8 from Paeoniae, 6 from Rhizoma, 2 from Rehmanniae, 4 from Atractylodes, 6 from Poria, 7 from Alisma, 9 from Amomum, and 15 from Hordei Fructus Germinatus.

For disease-associated targets, CKD-related targets numbered 14,616, 541, and 1,074 in GeneCards, OMIM, and DisGeNET, respectively, while depression-related targets were 12,902, 556, and 1,478, respectively. After removing duplicates, 15,075 CKD-related and 13,498 depression-related targets were retained. Intersection analysis using a Venn diagram revealed 69 shared targets between MDSS compounds and depression in CKD **(Figure 1)**, representing potential molecular targets through which MDSS may exert therapeutic effects.

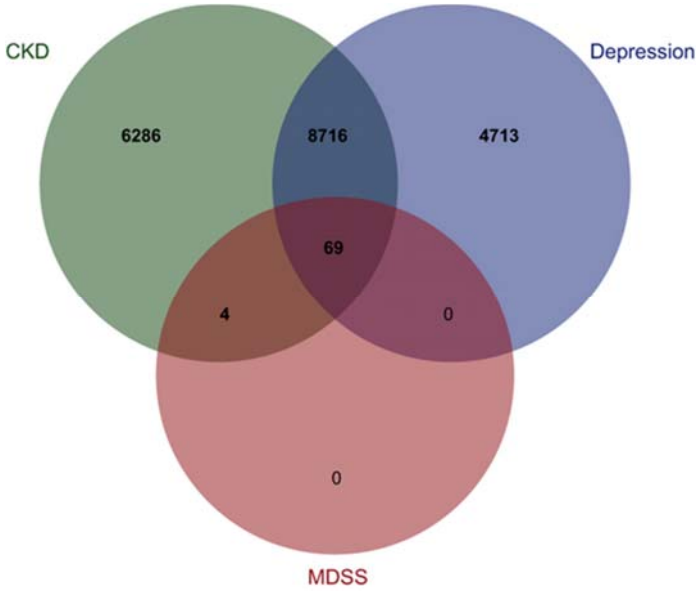


Figure 1. Venn diagram illustrating overlapping targets among CKD (green), Depression (blue), and MDSS (red).

Construction of the Herbal–Compound–Target Network and PPI Network for MDSS in Depression with CKD

The herbal compound target network was generated using Cytoscape software **(Figure 2)**. Network analysis identified six key bioactive compounds—β-sitosterol (MOL000358), stigmasterol (MOL000449), kaempferol (MOL000422), luteolin (MOL000006), myricanone (MOL002135), and sitosterol (MOL000359)—which exhibited the highest degree values among all 47 compounds, suggesting they may play central roles in mediating the antidepressant effects of MDSS in CKD patients.

The protein–protein interaction (PPI) network was constructed by inputting the 69 overlapping targets into the STRING database, resulting in a network with 66 nodes and 514 edges, and an average node degree of 31.15 **(Table 5).** Visualization and analysis of the PPI network in Cytoscape allowed identification of the top 10 hub targets using the degree-based ranking in the CytoHubba module **(Figure 3)**. Among these, IL6, ESR1, HSP90AA1, and CASP3 showed the highest degree scores, indicating that they may serve as key targets in the therapeutic effects of MDSS.

Table 5. Information about Potential Antidepressant Targets

Name	UniProt ID	Description	Degree
IL6	P05231	Interleukin-6	84
ESR1	P03372	Estrogen receptor 1	78
HSP90AA1	P07900	Heat shock protein HSP 90-alpha	74
CASP3	P42574	Caspase-3	72
EGFR	P00533	Epidermal growth factor receptor	70
PPARG	P37231	Peroxisome proliferator-activated receptor gamma	66
CCND1	P24385	Cyclin D1	64
RELA	Q04206	Transcription factor p65	58
MDM2	Q00987	E3 ubiquitin-protein ligase Mdm2	58
CASP8	Q14790	Caspase 8	54
ERBB2	P04626	Erb-B2 Receptor Tyrosine Kinase 2	54
MAPK8	P45983	Mitogen-Activated Protein Kinase 8	50
NFKBIA	P25963	NFKB Inhibitor Alpha	48
AR	P10275	Androgen Receptor	48
PGR	P06401	Progesterone Receptor	48
NR3C1	P04150	Nuclear Receptor Subfamily 3 Group C Member 1	46

CASP9	P55211	Caspase 9	44
MCL1	Q07820	Induced myeloid leukemia cell differentiation protein Mcl-1	42
RB1	P06400	RB Transcriptional Corepressor 1	42
GSK3B	P49841	Glycogen Synthase Kinase 3 Beta	42

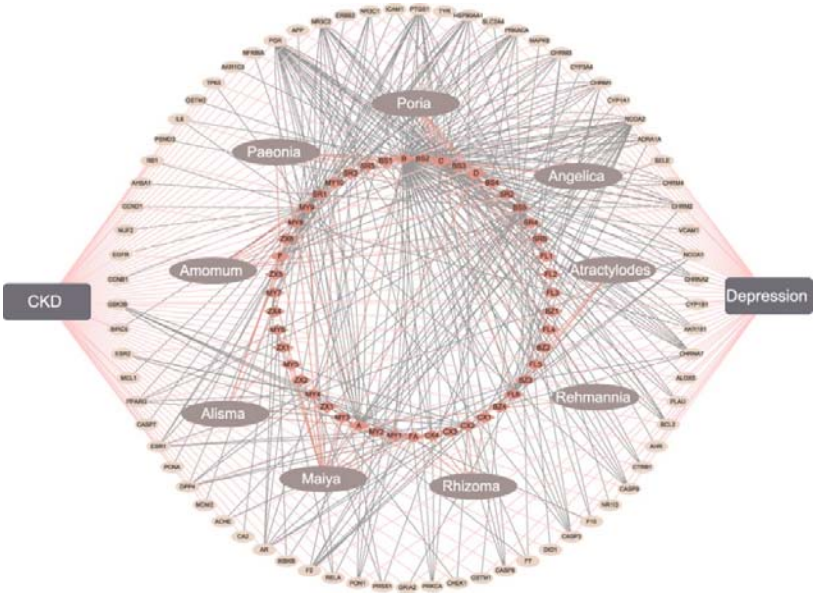
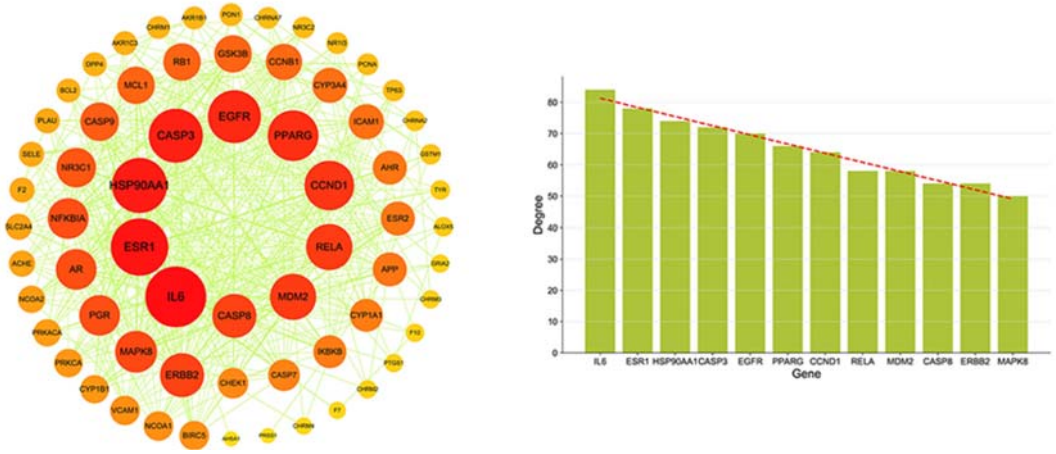


Figure 2. "Herbal–compound–target" network of MDSS for treating depression in CKD. Gray rectangles represent diseases, brown ovals indicate herbs, red quadrilaterals correspond to active compounds, and yellow ovals denote target genes.



a) b)

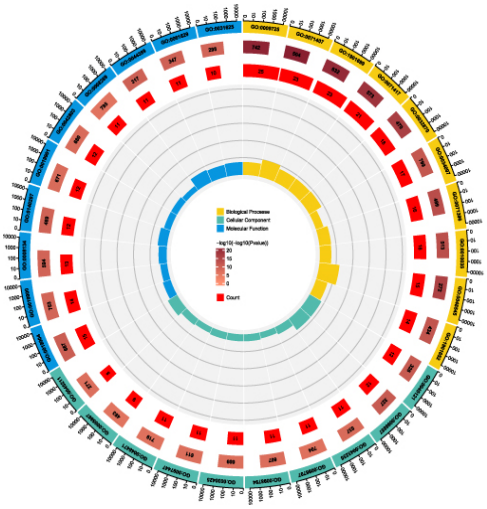
Figure 3. Protein–protein interaction (PPI) network diagram, where node size and color intensity reflect degree values (larger and redder nodes indicate higher degrees, smaller and yellow nodes indicate lower degrees) (a); and histogram of hub genes showing the degree values of the top 12 nodes in the PPI network (b).

GO and KEGG pathway analysis

Gene Ontology (GO) enrichment analysis was performed on the 69 targets derived from the PPI network, yielding 844 terms in total: 693 Biological Process (BP) terms, 51 Cellular Component (CC) terms, and 100 Molecular Function (MF) terms. The top 10 most significant terms for each category are presented in **Figure 4.**

KEGG pathway enrichment of the 69 genes identified 254 signaling pathways, with the 20 most enriched pathways visualized in **Figure 5**. The pathways showing the highest enrichment included Lipid and

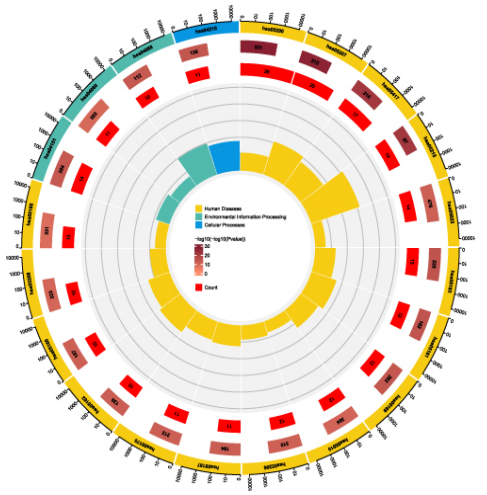
atherosclerosis, TNF signaling, PI3K-Akt signaling, Neuroactive ligand–receptor interaction, Pathways of neurodegeneration – multiple diseases, Apoptosis, and cancer-related pathways, among others.



ID	Description	ID	Description
GO:0009725	Response to hormone	GO:0045121	Membrane raft
GO:0071407	Cellular response to organic cyclic compound	GO:0098857	Membrane microdomain
GO:1901699	Cellular response to nitrogen compound	GO:0043235	Receptor complex
GO:0071417	Cellular response to organonitrogen compound	GO:0098797	Plasma membrane protein complex
GO:0032870	Cellular response to hormone stimulus	GO:0098794	Postsynapse
GO:0034097	Response to cytokine	GO:0030425	Dendrite
GO:0071396	Cellular response to lipid	GO:0097447	Dendritic tree
GO:0010035	Response to inorganic substance	GO:0048471	Perinuclear region of cytoplasm
GO:0048545	Response to steroid hormone	GO:0005667	Transcription regulator complex
GO:1901652	Response to peptide	GO:0045211	Postsynaptic membrane
GO:0019904	Protein domain specific binding	GO:0042803	Protein homodimerization activity
GO:0019900	Kinase binding	GO:0008289	Lipid binding
GO:0008134	Transcription factor binding	GO:0044389	Ubiquitin-like protein ligase binding
GO:0140297	DNA-binding transcription factor binding	GO:0061629	RNA polymerase II-specific DNA-binding transcription factor binding
GO:0019901	Protein kinase binding	GO:0031625	Ubiquitin protein ligase binding

Figure 4. GO term enrichment analysis.

The innermost circle represents the top 30 GO terms and their enrichment scores; the second circle shows the P-values for enrichment of the corresponding genes; the third circle indicates the number of enriched genes; and the outermost circle reflects the enrichment degree for each GO term, with yellow for Biological Processes, green for Cellular Components, and blue for Molecular Functions. GO, Gene Ontology.



ID	Description	ID	Description
hsa05200	Pathways in cancer	hsa05167	Kaposi sarcoma -associated herpesvirus infection
hsa05207	Chemical carcinogenesis - receptor activation	hsa05170	Human immunodeficiency virus 1 infection
hsa05417	Lipid and atherosclerosis	hsa05162	Measles
hsa05215	Prostate cancer	hsa05160	Hepatitis C
hsa05022	Pathways of neurodegeneration - multiple diseases	hsa05208	Chemical carcinogenesis - reactive oxygen species
hsa05163	Human cytomegalovirus infection	hsa05165	Human papillomavirus infection
hsa05161	Hepatitis B	hsa04151	PI3K-Akt signaling pathway
hsa05169	Epstein-Barr virus infection	hsa04080	Neuroactive ligand-receptor interaction
hsa05010	Alzheimer disease	hsa04668	TNF signaling pathway
hsa05206	MicroRNAs in cancer	hsa04210	Apoptosis

Figure 5. KEGG pathway enrichment analysis.

The innermost circle shows the top 20 KEGG pathways and their enrichment scores; the second circle indicates the P-values for enrichment; the third circle represents the number of enriched pathways; and the outermost circle reflects the enrichment degree of each KEGG term, with yellow representing Human Diseases, green for Environmental Information Processing, and blue for Cellular Processes. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Molecular docking

Ligand–target binding results in a conformationally stable structure, where lower binding energies indicate greater stability. A binding energy below 0 kcal/mol suggests spontaneous ligand–protein interaction, with more negative values indicating stronger binding affinity and a higher likelihood of interaction. Docking analyses were conducted between six bioactive compounds (β-sitosterol, stigmasterol, kaempferol, luteolin, myricanone, and

sitosterol) and four key target proteins, with the lowest binding energies presented in a heat map **(Figure 6)**. Electrostatic interactions and van der Waals forces were the primary contributors to ligand–protein binding. β-sitosterol bound to CASP3 with the lowest energy (−9.56 kcal/mol), while sitosterol and kaempferol also showed strong binding to CASP3 (−9.5 kcal/mol and −9.49 kcal/mol, respectively). Representative molecular docking interactions are illustrated in **Figure 7**, where the protein receptor is shown in purple, the ligand in green, and hydrogen bonds are indicated by yellow dashed lines, demonstrating stable ligand–receptor binding.

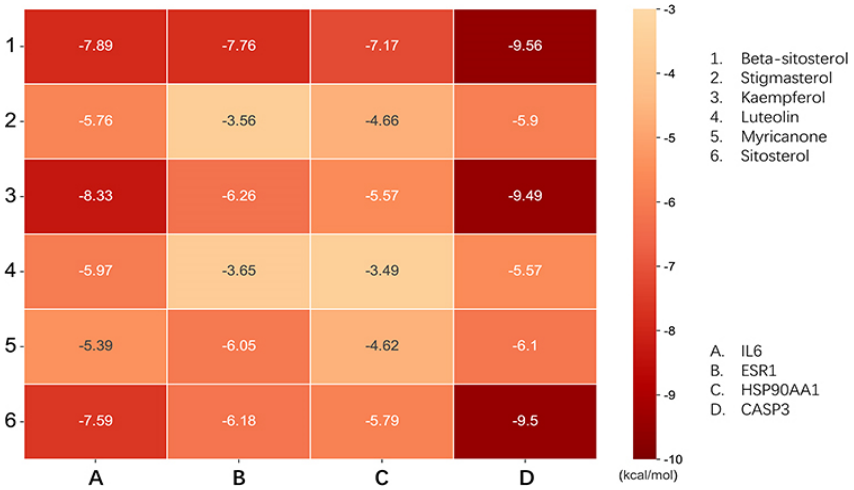


Figure 6. Molecular docking results (1 kcal = 4.184 kJ) for key target proteins: (A) IL6; (B) ESR1; (C) HSP90AA1; (D) CASP3.

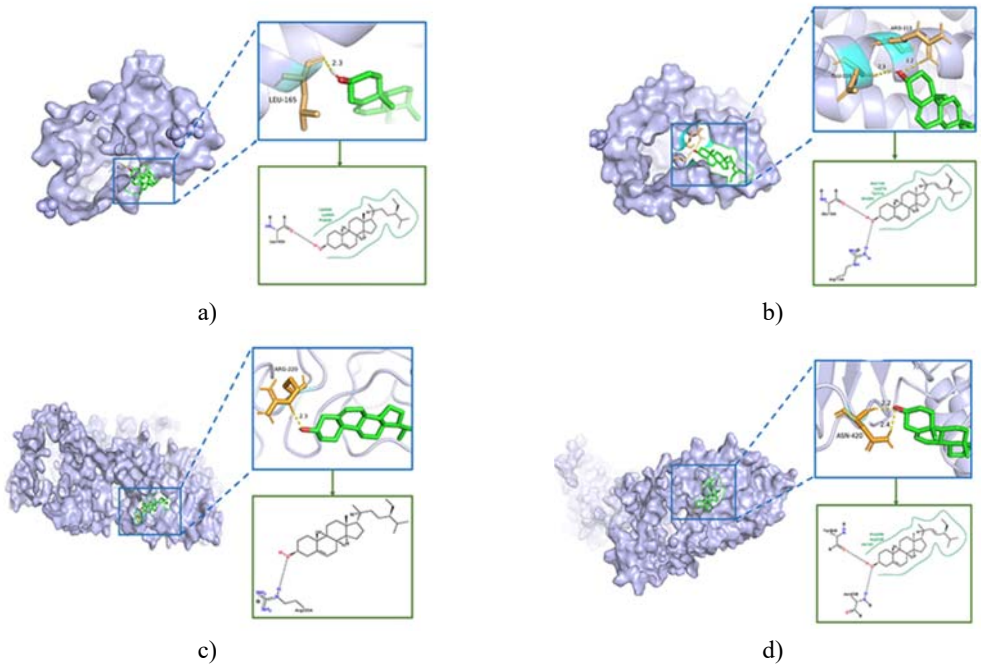


Figure 7. Visualization of molecular docking results, shown in both 3D and 2D representations: (a) IL6–β-sitosterol; (b) IL6–stigmasterol; (c) ESR1–β-sitosterol; (d) ESR1–β-sitosterol.

Results and Discussion

Depression frequently occurs in patients with CKD, markedly impairing their quality of life and survival outcomes. Conventional antidepressant therapies for CKD patients remain controversial due to safety concerns, and many patients fail to achieve optimal results. Previous studies have demonstrated the antidepressant efficacy

of DSS [7, 8]. MDSS, a modified version of DSS designed to nourish the kidneys, has been applied to treat depression, yet its clinical efficacy and underlying mechanisms have not been fully elucidated.

In this study, MDSS was administered for the first time as a compound herbal therapy for depression in CKD patients. Consistent with the effects of DSS in general populations [7, 24], MDSS was enhanced with kidney-beneficial herbs and showed notable efficacy in alleviating depressive symptoms in CKD patients. At therapeutic doses, MDSS did not worsen renal function, elevate hepatic transaminases, or cause hyperkalemia. Moreover, the regimen supports kidney and liver health, relieves liver stress, calms the mind, and effectively reduces depressive symptoms, confirming its preliminary clinical efficacy and safety.

Network pharmacology analysis further elucidated the potential mechanisms of MDSS, identifying six key bioactive compounds—β-sitosterol, stigmasterol, kaempferol, luteolin, myricanone, and sitosterol. Both CKD and depression share pathophysiological features involving oxidative stress and inflammation [25–27]. β-sitosterol can regulate the glutathione redox cycle by preventing intracellular ROS accumulation [28] and shows a strong affinity for estrogen receptors, enhancing antioxidant defense either directly or by stimulating antioxidant enzymes [29]. Additionally, β-sitosterol inhibits phosphorylation and degradation of IκB, suppressing NF-κB and ERK phosphorylation, which reduces the expression of inflammatory mediators such as iNOS, TNF-α, and COX-2 [30]. Stigmasterol exhibits similar anti-inflammatory and antioxidant effects. Kaempferol has been shown to inhibit LPS-stimulated NO and TNF-α production [31] and suppress PI3K/Akt phosphorylation, thereby mitigating cellular inflammation [32]. Luteolin possesses antioxidant, anti-apoptotic, and anti-inflammatory properties [33] and has demonstrated therapeutic effects in chronic inflammatory disorders, acute kidney injury, neurological diseases, and cancer [34, 35]. Myricanone also exhibits anti-inflammatory and antioxidant actions [36]. Collectively, these multiple compounds likely act synergistically, providing anti-inflammatory, antioxidant, and anti-apoptotic effects that form the pharmacological basis for MDSS in treating CKD-associated depression.

PPI network analysis highlighted IL6, ESR1, HSP90AA1, CASP3, EGFR, PPARG, CCND1, RELA, MDM2, and CASP8 as key targets, which are predominantly involved in inflammation, oxidative stress, and apoptosis—consistent with the pathophysiology of CKD and depression [37, 38]. Molecular docking of four core targets (IL6, ESR1, HSP90AA1, CASP3) with the principal active compounds revealed strong binding, particularly to IL6 and CASP3. IL6 is a central mediator of inflammation and immune responses, and its levels are elevated in depressed patients [39]. IL6 gene polymorphisms influence depressive symptom severity [40], and pro-inflammatory factors, including IL6, contribute to kidney diseases such as CKD [41, 42]. CASP3, a cysteine protease, is involved in depression and kidney disease through apoptotic and inflammatory pathways [43, 44]. These targets represent crucial nodes for MDSS's therapeutic effects in CKD patients with depression.

GO and KEGG enrichment analysis indicated that the PI3K-Akt signaling pathway, neuroactive ligand-receptor interaction, TNF signaling pathway, and apoptotic pathway were the most relevant pathways through which MDSS may act in CKD-associated depression, alongside pathways linked to human diseases. Among these, 14 genes were enriched in the PI3K-Akt signaling pathway, a crucial intracellular immune and inflammatory pathway, with PI3K and Akt as central proteins. Akt not only modulates TNFα and peroxisome proliferator-activated receptor delta (PPARβ/δ) via NF-κB activation but also phosphorylates murine double minute 2 (MDM2) to trigger p53 degradation and regulates the GSK3 heterodimer at its conserved N-terminal site, influencing apoptosis and glucose metabolism [45]. The anti-inflammatory and antioxidant effects of β-sitosterol are also mediated through this pathway. Additionally, enrichment of the neuroactive ligand-receptor interaction pathway, relevant to MDSS's antidepressant effects, is associated with neuronal differentiation processes, including hippocampal glial cell activation and glioma modulation [46–48], and can act synergistically with the PI3K-Akt pathway to suppress inflammatory responses [49]. The enrichment of TNF and apoptotic pathways further supports that MDSS exerts effects via multiple pathways and targets, integrating mechanisms of inflammation, oxidative stress, and apoptosis.

Limitations of this study include the absence of a positive control group, the relatively small sample size, and the short follow-up duration. Future research would benefit from multicenter trials with larger cohorts, extended treatment periods, and comparisons to standard therapies. Notably, this study represents the first clinical investigation of a compound herbal formula for depression in CKD patients, which led to the inclusion of participants with relatively good adherence and stable general condition. Nonetheless, the findings demonstrate the efficacy of MDSS in improving depressive symptoms with minimal impact on renal function and fewer side effects compared with conventional antidepressants, suggesting its potential as an alternative therapeutic option for depression in CKD. Moreover, the study employed a network pharmacology approach to preliminarily explore

the molecular mechanisms and targets of MDSS in CKD-associated depression, although in vivo validation in animal models has not yet been conducted due to the complexity of modeling depression and subjective symptom evaluation. These results provide a foundation for future mechanistic research.

Conclusion

Administration of MDSS for six weeks alleviated depressive symptoms in CKD patients. Network pharmacology analysis identified β-sitosterol, stigmasterol, kaempferol, luteolin, and myricanone as the key active compounds, likely acting on targets such as IL6, ESR1, HSP90AA1, and CASP3. The underlying pharmacological effects of MDSS are likely mediated through regulation of oxidative stress, inflammation, and apoptosis.

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