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ORIGINAL RESEARCH

Preliminary Exploration of Clinical Efficacy and Pharmacological Mechanism of Modified Danggui-Shaoyao San in the Treatment of Depression in Patients with Chronic Kidney Disease

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Background: Depression in Chronic Kidney Disease (CKD) seriously affects the prognosis of patients and Modified Danggui-Shaoyao-San (MDSS) is based on the traditional Chinese formula Danggui-Shaoyao-San (DSS) for the treatment of depression, which is further optimized. The aim of this study was to evaluate the clinical efficacy and safety of MDSS for the treatment of depression in CKD, and to explain the molecular mechanism of MDSS for the treatment of depression in CKD through pharmacology and molecular docking.

Methods: 62 patients were randomly divided into treatment group (treated with MDSS) and control group (treated with placebo) and assessed by Hamilton Depression Scale, and the primary outcome was to evaluate the efficacy of MDSS in improving depressive symptoms and the effect on liver and kidney function, electrolytes. In addition, we identified the core compounds and potential targets of MDSS through the TCMSP database. The GeneCards, OMIM and Disgenet databases were then used to identify molecular targets for CKD and depression. The target protein–protein interaction network was built using STRING database. Core targets were analyzed by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Molecular docking was used to verify the relationship between core active compounds and proteins.

Results: Clinical results showed that CKD patients in the MDSS group had significantly improved depressive status with no significant adverse effects. By network pharmacology analysis, we found that the compound-target network mainly contained 47 compounds and 69 corresponding targets. 844 terms were analyzed by GO enrichment, and 254 signaling pathways in KEGG. Molecular docking showed that the top active compounds had high affinity with four targets.

Conclusion: We preliminarily investigated the efficacy of MDSS in the treatment of depression in CKD and revealed the characteristics of multiple compounds and multiple targets in MDSS.

Keywords: Modified Danggui-Shaoyao San, depression, chronic kidney disease, clinical efficacy, network pharmacology

Introduction

Depression is a leading contributor to the overall global burden of disease,[1] and as a common comorbidity in patients with chronic kidney disease (CKD), depression is five times more prevalent in patients with CKD than in the general population.[2] Also, it exacerbates clinical symptoms associated with CKD, decreases the quality of life and medication adherence of patients,[3] as well as significantly increases cardiovascular events and deaths.[4] However, the current major approach to the therapy of depression in comorbid CKD is mostly western pharmacotherapy, and these medications are often associated with serious hepatic and renal toxicity.[5] On the one hand, the renal dysfunction of CKD patients

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exacerbates this adverse effect; on the other hand, physicians may fear the adverse effects of these drugs on the renal function of CKD patients and thus reduce the dose of antidepressants or abandon antidepressant treatment for CKD patients.[6] This ultimately limits the treatment strategy for depression in CKD patients.

Danggui-Shaoyao-San (DSS) originated from the earliest Chinese monograph on the diagnosis and treatment of miscellaneous diseases, "Synopsis of Golden Chamber", and is a traditional compound Chinese medicinal preparation composed mainly of Angelica and Paeoniae, which has been used since ancient times to nourish the heart and calm the mind, and modern studies have confirmed that this formula can significantly improve depression.[7,8] In recent years, famous Sichuan traditional Chinese medicine practitioners combined the clinical herbal characteristics and clinical medication experience to further improve the basis DSS of adding diuretic and kidney-nourishing herbs such as Rehmanniae, Poria and Amomum, etc., which nourish the liver and kidney, to form Modified Danggui-Shaoyao-San (MDSS). In the MDSS formula, Rehmanniae enters the kidneys and diuretic, while Angelica enters the liver, nourishes yin, nourishes blood, activates blood circulation, and invigorates blood circulation, which has become a famous local formula for the treatment of depression in CKD. However, there are no large-scale controlled clinical studies to confirm the clinical efficacy and pharmacological mechanism of action of MDSS.

Therefore, this study aimed to fill this gap by exploring the clinical efficacy of the herbal formulation MDSS in the treatment of depression in patients with chronic kidney disease. The study investigated the clinical efficacy and safety of MDSS in treating depression in CKD patients through a 6-week single-blind randomized clinical trial, and the molecular mechanism of action of MDSS in treating depression in CKD patients was further explored with network pharmacology combined with molecular docking, which provides valuable evidence for future basic research and clinical application.

Materials and Methods

Clinical Efficacy Study

This study was performed using a randomized, controlled, single-blind study design. The trial protocol was approved by the Second Clinical Medical Institution of North Sichuan Medical College (Grant: 2020–134) and directed steadily with the Declaration of Helsinki and the resulting amendments. The preliminary was enrolled at the Chinese clinical preliminary library (www.chictr.org.cn; enlistment number: ChiCTR2100041867) preceding the review. Written informed consents were obtained from all patients or family authorized before enrollment. Patients were educated that they were allowed to pull out from the preliminary whenever with practically no unfavorable impact on their relationship with the supplier and their treatment.

Inclusion and Exclusion Criteria

The enrolled patients met the diagnostic criteria for DSM-5 depression published by the American Psychiatric Association 2013,[9] and according to the KIDGO criteria for CKD stages 1–3 established by the American Society of Nephrology.[10] The diagnostic criteria of Traditional Chinese Medicine refer to the identification criteria of kidney deficiency and liver depression in the "Guidelines for the Treatment of Common Diseases in Internal Medicine".[11] The primary symptom is depression of output, but also interest is absent, irritability, slow thinking, fatigue, insomnia, forgetfulness, loss of libido, and decreased appetite; secondary symptoms include disappointment with output, laziness and retreat, soreness and weakness of the waist and knees, distension and swelling of the chest, chest tightness and chest discomfort, shortness breath, dull face, light or dark tongue, white tongue coating, and sunken string pulse.

Inclusion criteria: i) the patients met the above diagnostic and identification criteria; ii) were of age 18–65 years; iii) met the Chinese and Western medical diagnostic criteria for depression diagnosis; iv) had a Hamilton Depression (HAM-D) score ≥7; v) gave informed consent and voluntarily participated in this study; Exclusion criteria: i) unstable vital signs; ii) CKD stage 5D dialysis or CKD stage 3–4 oliguria or anuria; iii) severe aphasia or loss of recognition and inability to communicate; iv) certain schizophrenic symptoms; v) depressive symptoms associated with other psychiatric disorders (such as bipolar disorder, obsessive-compulsive disorder, schizophrenia, etc.) as clinically diagnosed by Western medicine; vi) evidence of an etiological relationship between anxiety disorders and a physical disease; vii) known alcohol or drug dependence. Patients who met at least one of the above criteria were excluded.

Intervention

A total of 62 patients admitted from December 2020 to December 2021 at Santai County Traditional Chinese Medicine Hospital, who met the inclusion criteria, were selected and divided into treatment group (n=31) and control group (n=31) using the random number table method. Those included in the treatment group were given MDSS (15g of Rehmanniae, 15g of Angelicae, 15g of Paeoniae, 20g of Rhizoma, 15g of Atractylodes, 15g of Poria, 12g of Alisma, 10g of Amomum, 15g of Hordei Fructus Germinatus for one dose, 8 doses were prepared; crushed into powder, made into pills and available for application) 20 g/day for 6 weeks in the early morning of the second day after admission. The control group was given a placebo at the same time points for 6 weeks.

Outcome of Clinic Part

We examined the effect of MDSS compared to placebo on improvement in depressive symptoms at baseline and at weeks 2, 4 and 6. The HAM-D score is the most used scale in the clinical evaluation of depression.[12] The total score better reflects the severity of depression, whereby the lower the total score, the less severe the condition, and the higher the total score, the more severe the condition. The primary outcome was the change in HAM-D scores from baseline to each time point; and secondary outcome measures: i) partial responders (25–50% reduction in HAM-D scores), ii) responders (≥50% reduction in HAM-D scores), iii) remitters (HAM-D scores ≤7). iv) Response and remission rates between the two groups.[13] v) Blood creatinine (CR), blood urea nitrogen (BUN), serum alanine aminotransferase (ALT), glutamate aminotransferase (AST), and blood potassium (K+) were also measured at baseline and 6 weeks after the intervention to assess the effect of MDSS on liver and kidney function and electrolytes. At a similar time, adverse reactions due to the drugs were monitored.

Statistical Analysis

Continuous variables were expressed as means and standard deviations (SD) and categorical variables were expressed as frequencies and percentages. Categorical variables were compared using the chi-square test and Fisher's exact test where appropriate. Data from each group of patients before and after the intervention were counted using a paired *t*-test, and an independent *t*-test was used to compare the mean of continuous variables between treatment groups. P value less than 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 19 Software (IBM Company, USA).

Collection of Component Targets of MDSS

MDSS included Rehmanniae, Angelicae, Paeoniae, Rhizoma, Atractylodes, Poria, Alisma, Amomum, Hordei Fructus Germinatus were mainly collected from TCMSP (http://tcmspw.com/tcmsp.php).[14] The corresponding compounds and targets were obtained with Oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18.[15] The screened proteins were converted to the human gene by the Uniprot database (https://www.uniprot.org/).[16]

Prediction of Targets Associated with Depression in CKD

Genes related to depression in CKD were obtained from Genecards (https://www. genecards.org/),[17] OMIM (https://omim.org/)[18] and Disgenet (https://www.disgenet.org/home/)[19] by searching "CKD" and "Depression". All genes were merged and duplicate data were removed to obtain candidate targets for the treatment of CKD combined with depression. The common targets of MDSS, CKD and depression-related genes were intersected with the Venn map. The intersected genes were the target genes of MDSS for the treatment of depression in CKD.

Construction of Compound-Target and Protein–Protein Interaction (PPI) Network

The bioactive components of MDSS and intersected genes were imported into Cytoscape version 3.8.2 to construct the compound-target network of MDSS for the treatment of depression in CKD. The intersected genes were imported into String database (https://string-db.org/),[20] the species was defined as "Homo sapiens", and discrete targets were Hidden. The PPI network was constructed and analyzed by Cytoscape.

Gene Ontology (GO) and Pathway Analysis

We used the Metascape database (http://metascapape.org)[21] for Gene Ontology (GO) biological process enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The intersected genes were imported into the Metascape database. The selected identifier was set to the official gene symbol. The list type was set to gene list, and the species was limited to "Homo sapiens", $P < 0.05$ was saved as screening criteria for biological processes and signaling pathways. Go analysis and KEGG pathway analysis were performed on the intersected genes.

Molecular Docking

Candidate proteins of a higher degree in the PPI network were considered key protein targets, and they were molecularly docked to active ingredient compounds. The UniProt database was used to find the UniProt ID of the key protein targets. The 3D protein structures of key proteins were downloaded from the RCSB PDB platform (http://www.rcsb.org/)[22] based on the UniPort ID, and disability and water molecules were removed using Pymol 2.4.0 software. The 3D structures corresponding to the active ingredient compounds were obtained from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/).[23] AutodockTools 1.5.6 software defined the location of the active pockets and molecular docking was completed using Autodock Vina 1.1.2 software, and docking was performed via Pymol 2.4.0 software for 3D structure visualization and Proteins plus (https://proteins.plus/) for 2D results visualization.

Results

Basic Data of the Patients

In this clinical study, 62 subjects were recruited and randomized in a 1:1 ratio into an experimental and placebo group. There were 14 males and 17 females in the treatment group with a mean age of 47.0 ± 10.5 years. There were 16 males and 15 females in the placebo group with a mean age of 48.3 ± 10.5 years. After comparing gender, age, HAM-D score and biochemical indices before treatment between the two groups, the results showed no significant differences between the two groups, as shown in Table 1.

Outcomes and Side Effects

HAMD scores at baseline were not significantly different between the treatment and control groups (14.83 ± 4.48 vs 13.58 ± 3.47, respectively, $p = 0.227$). Although HAMD scores after 6 weeks, the difference between groups was not significant ($p = 0.114$). However, by comparing the HAMD scores in the treatment group before and after the intervention, there was a significant decrease in HAMD scores from week 2 from baseline, with a significant difference ($p = 0.001$), and this trend was maintained until the end of the 6-week period of the research. However,

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.

the scores in the control group were not significantly different from baseline after treatment. From baseline to 2 weeks later, HAMD scores diminished by 1.16 ± 1.75 and 0.00 ± 2.03 in the treatment and control groups, separately, with a significant difference between the groups (P= 0.019); at about 4 weeks of treatment, HAMD scores diminished to 2.39 ± 2.74 and - 0.03 ± 3.31 in the treatment and control groups, individually, with a significant difference between the groups (P=0.003). At about 6 weeks of treatment, HAMD scores diminished to 3.39 ± 3.29 and 0.16 ± 4.53 in the treatment and control groups, separately, with a significant difference between the groups (P= 0.002), in Table 2. We compared the proportion of patients in the two groups who achieved partial responders, responders, and remission in their HAMD scores after the intervention; the higher the proportion, the better the outcome. The proportions of partial responders were 35.5% and 25.8%, the proportions of responders were 3.2% and 0%, and the proportions of those with HAMD scores ≤7, or remission, were 22.6% and 12.9%, respectively. As shown in Table 3.

There was no significant difference in BUN, CR, AST, ALT, and K+ after the intervention between the treatment group and the control group, and there was no significant difference in these indices before and after the intervention when comparing within the group (Table 4). It indicated that no further deterioration of renal function and adverse effects such as abnormal liver function and hyperkalemia occurred after the MDSS application. 2 patients in the treatment group experienced constipation and 1 patient experienced sweating during the trial period, and 2 patients in the control group experienced headache, with no major adverse events and no deaths in both groups.

Screening of Bioactive Compounds and Related Targets

A total of 47 compounds from 9 herbs with OB≥ 30% and DL≥ 0.05 were screened, including 2 compounds from Angelicae, 8 compounds from Paeoniae, 6 compounds from Rhizoma, 2 compounds from Rehmanniae, 4 compounds

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: Data are shown as mean (SD). P1-value (by using paired *t*-test) represents the comparison before and after intervention in treatment group; P2-value (by using paired *t*-test) represents comparison before and after intervention in control group; P3-value (by using independent-samples *t*-test) represents the comparison between treatment and control groups after intervention. P-value of <0.05 was considered statistically significant, as indicated by bold typeface.

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 shown as mean (SD). P-value of <0.05 was considered statistically significant.

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 shown as mean (SD). P1-value (by using paired *t*-test) represents comparison before and after intervention in treatment group; P2-value (by using paired *t*-test) represents the comparison before and after intervention in control group; P3- value (by using independent-samples *t*-test) represents the comparison between treatment and control groups after intervention. P-value of < 0.05 was considered statistically significant.

from Atractylodes, 6 compounds from Poria, 7 compounds from Alisma, 9 compounds from Amomum and 15 compounds contained in Hordei Fructus Germinatus, as shown in Supply Table 1.

For disease targets, 14,616, 541, and 1074 CKD-related targets were obtained in Genecards, OMIM, and Disgenet, respectively, and 12,902, 556, and 1478 depression-related targets, respectively. After removing duplicate targets, 15,075 CKD-related target targets were identified, and depression-related disease targets 13,498. After mapping disease targets and MDSS-related targets in a Venn diagram, 69 overlapping targets of depression in CKD with the treatment of MDSS were accessed (Figure 1).

Construction of Herbal-Compound-Target Network and PPI Network of MDSS in the Treatment of Depression in CKD

We obtained the herbal-compound-target network using the analytical network tool in Cytoscape software (Figure 2). After network analysis, we found that β-sitosterol (MOL000358), Stigmasterol (MOL000449), Kaempferol

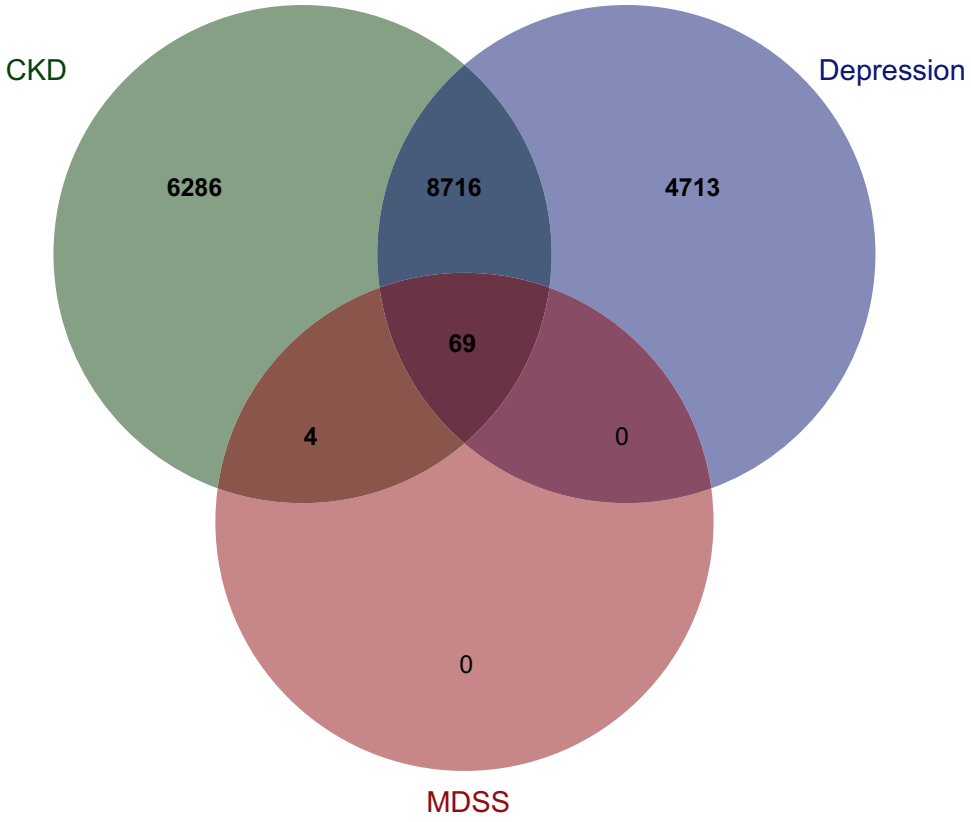


Figure 1 Venn diagram summarizing differential targets of CKD (green), Depression (blue) and MDSS (red).

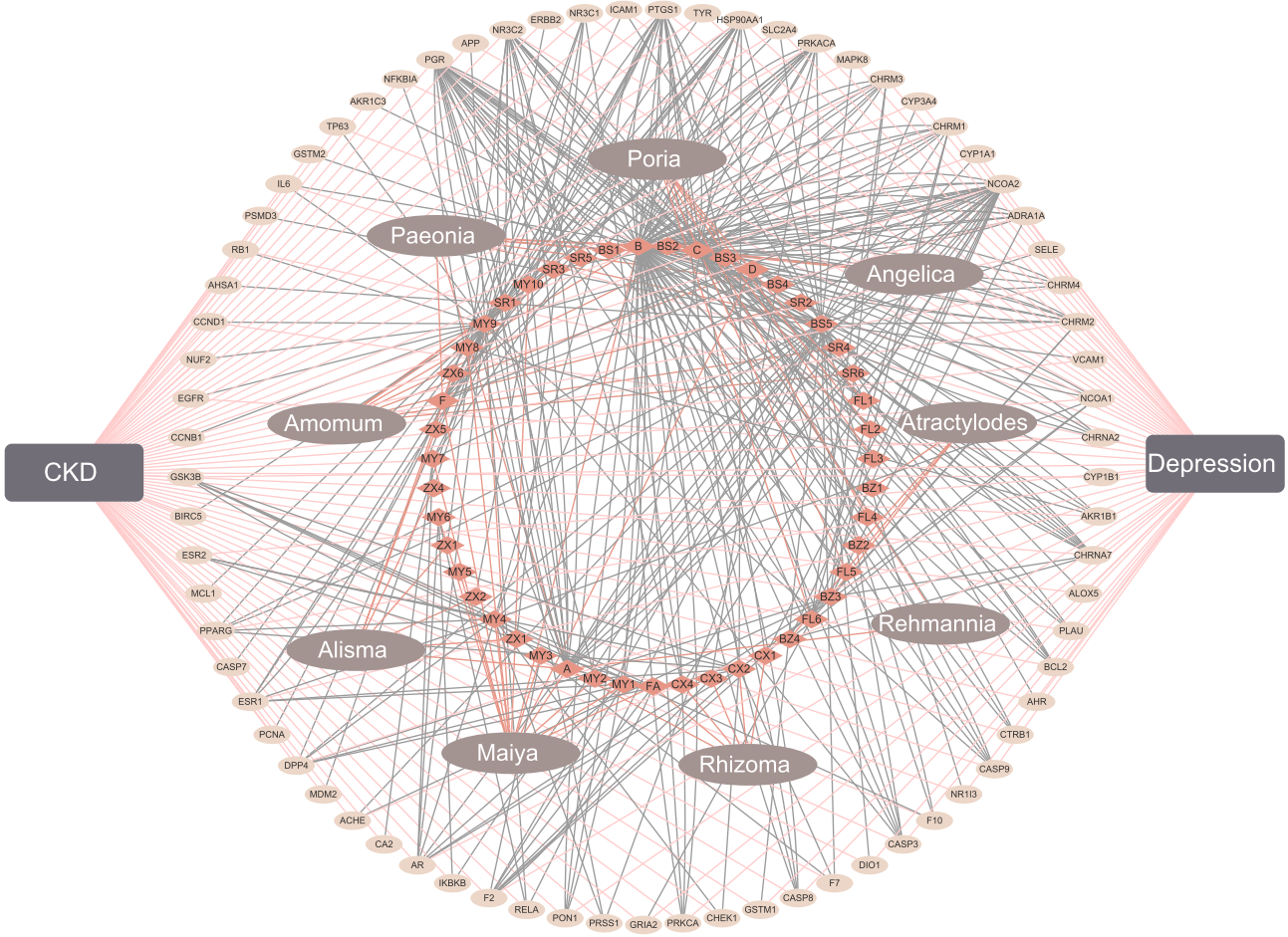


Figure 2 The "herbal-compound-target" network diagram of MDSS in the treatment of Depression in CKD. (Gray rectangle represents disease; brown oval represents herbal medicine; red quadrilateral represents active ingredient; yellow oval target gene).

(MOL000422), Luteolin (MOL000006), Myricanone (MOL002135) and Sitosterol (MOL000359) the 6 important active substances with the top degree values among all 47 compounds. These 6 substances may play the most important role in the treatment of depression in CKD patients. This result suggested that MDSS exerted antidepressant effects in CKD patients mainly through these compounds. A PPI network was worked by contributing 69 covering targets to the STRING database. The objective organization has 66 hubs and 514 edges, with a typical hub level of 31.15 (Table 5.). The PPI network information was envisioned and dissected by the Cytoscape, and the main 10 center targets were chosen by involving the plummeting request of degree in the Cytohubba module (Figure 3). Because of the most significant level scores got, IL6, ESR1, HSP90AA1, and CASP3 might assume pivotal parts in the treatment of MDSS.

GO and KEGG Analysis

GO analysis was conducted on 69 targets obtained from the PPI network to screen out the top 10. 844 GO terms were identified, including 693 Biological Processes (BP) terms, 51 Cellular Component (CC) terms and 100 Molecular Function (MF) terms. Figure 4 shows the top 10 most important terms for BP, CC, and MF. Based on the KEGG pathway analysis of 69 genes, a total of 254 signal pathways were identified. And top 20 enriched pathways were visualized in Figure 5. The most significantly enriched pathways were the Lipid and atherosclerosis, TNF signaling pathway, PI3K-Akt signaling pathway, Neuroactive ligand-receptor interaction, Pathways of neurodegeneration - multiple diseases, Apoptosis, pathways in cancer, etc.

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

Molecular Docking

When a ligand binds to a target to form a conformationally stable structure, the lower the energy, the more stable the structure. When the binding energy is <0 kcal/mol, the molecular ligand can spontaneously bind to the protein receptor. If the binding energy is <0 kcal/mol or lower, it indicates a stronger binding ability and a higher probability of interaction between them. We calculated the precision of docking between the six bioactive ingredients (Beta-sitosterol, Stigmasterol, Kaempferol, Luteolin, Myricanone, and Sitosterol) and four potential target proteins, and the lowest docking results were presented in a heat map (Figure 6). Electrostatic force and van der Waals forces were the main

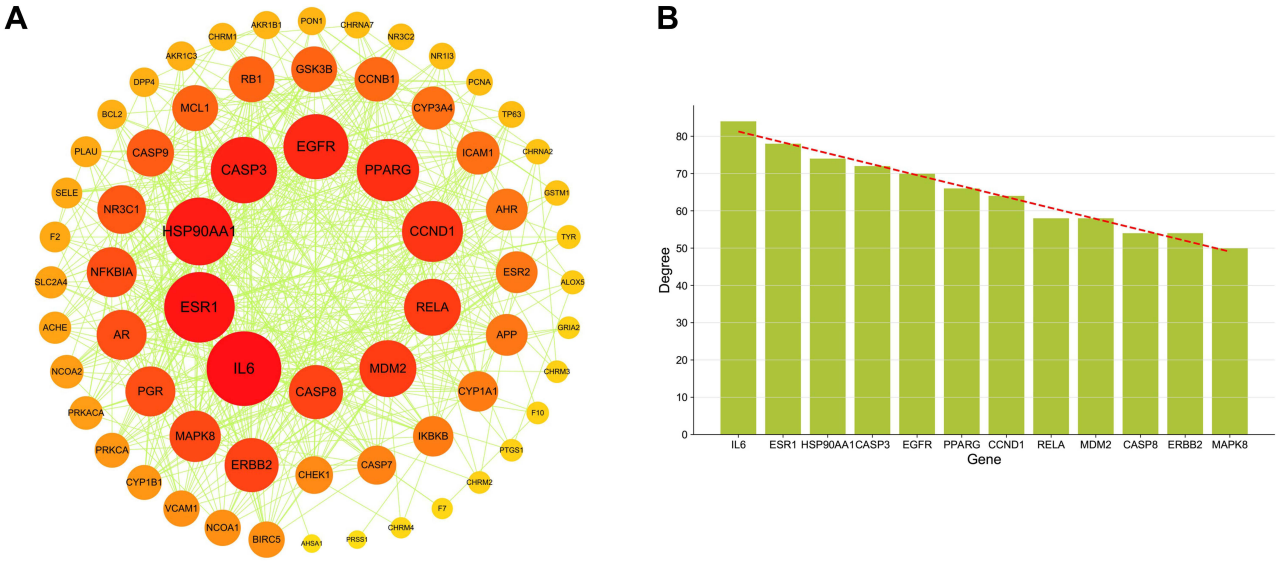
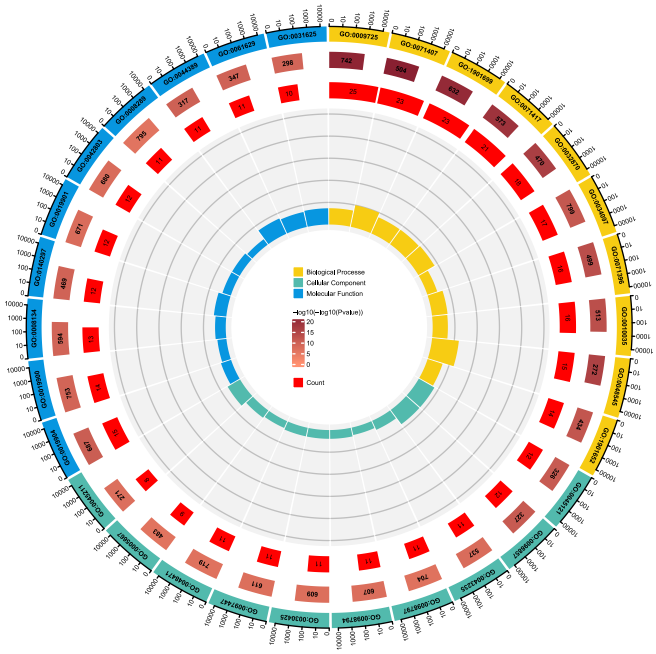
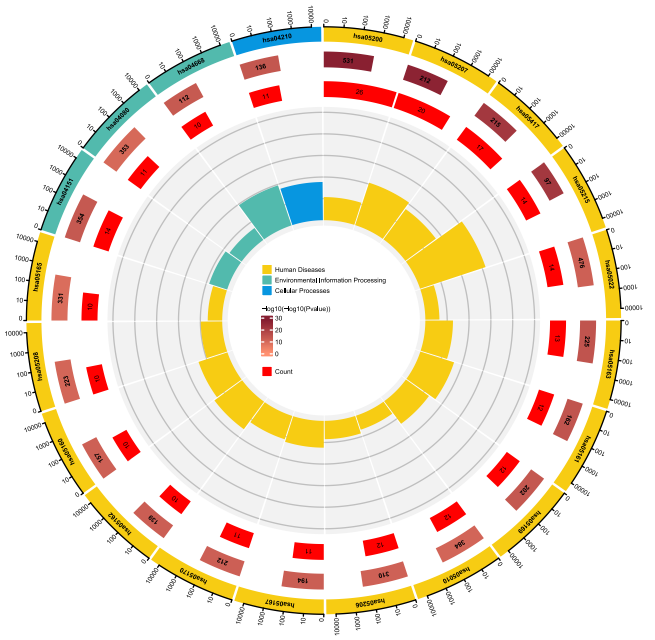


Figure 3 PPI network diagram (the sizes and colors of the nodes and lines are illustrated from large to small and red to yellow in descending order of degree values) (**A**); and Hub genes histogram (the degree value of the top 12 genes in the PPI network) (**B**).



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 analysis. The first lap indicates the top 30 GO terms, and the degree of enrichment corresponds to the inner circle; The second lap indicates the P values for enrichment for specified gene; The third lap indicates the count of enriched genes; The fourth lap indicates the enrichment degree of each GO term (yellow is Biological Processes; green is Cellular Component; blue is Molecular Function). 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 term analysis. The first lap indicates the top 20 KEGG terms, and the degree of enrichment corresponds to the inner circle. The second lap indicates the P values for enrichment. The third lap indicates the count of enriched signal pathways. The fourth lap indicates the enrichment degree of each KEGG term (yellow is Human Diseases; green is Environmental Information Processing; blue is Cellular Processes). KEGG, Kyoto Encyclopedia of Genes and Genomes.

forces between ligand and target protein. Beta-sitosterol and CASP3 were docked with the lowest binding energy (−9.56 kcal/mol), Sitosterol and Kaempferol were docked with CASP3 (−9.5 kcal/mol and −9.49 kcal/mol) with the second and third binding energies, respectively. The binding energies of Sitosterol and Kaempferol docked with CASP3 (−9.5 kcal/

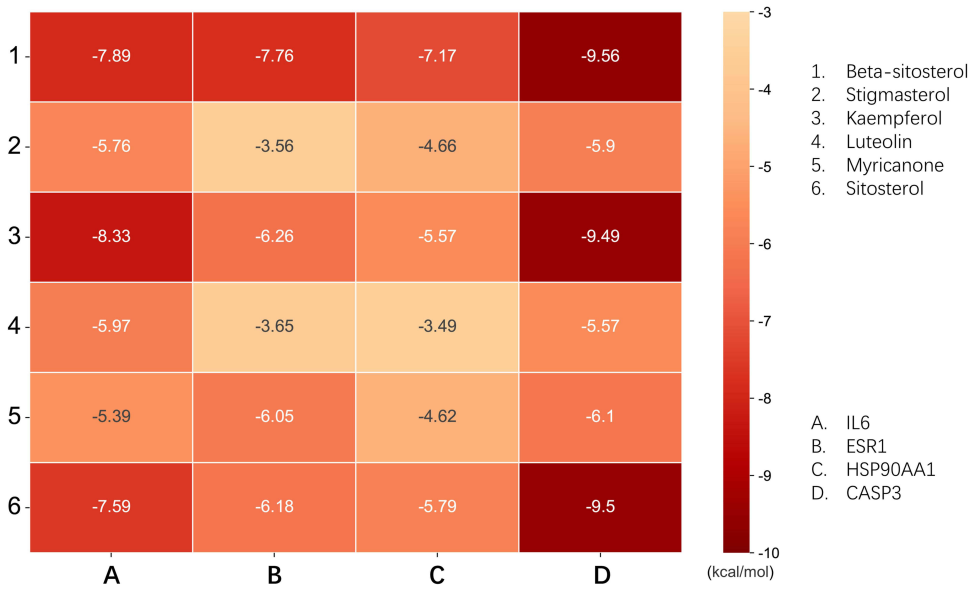


Figure 6 Results of molecular docking (1 kcal=4.184 kJ). (**A**) IL6; (**B**) ESR1; (**C**) HSP90AA1; (**D**) CAHP3.

mol and −9.49 kcal/mol) were ranked second and third. Parts of the molecular docking binding patterns were shown in Figure 7, where purple was the protein receptor, green was the molecule ligand, and the yellow dashed line was the hydrogen bond formed, indicating a more stable binding between the ligand and the receptor.

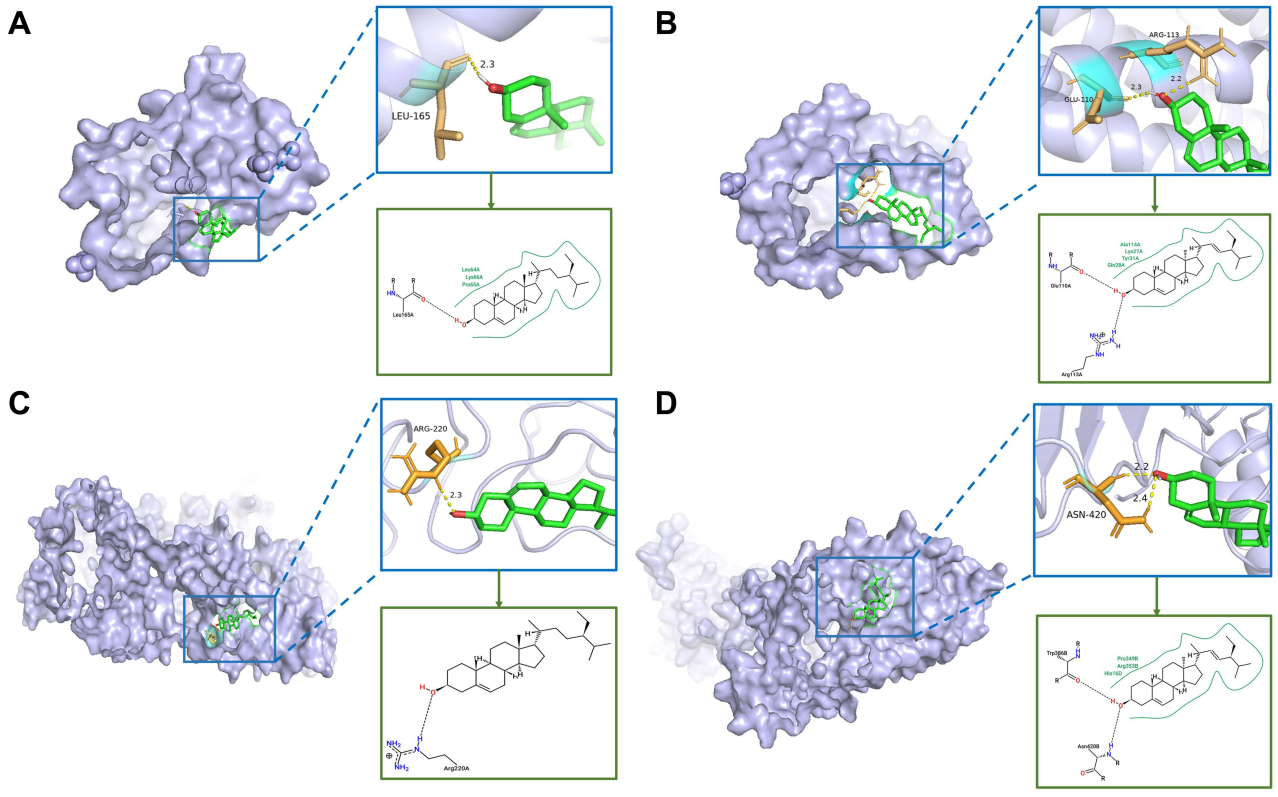


Figure 7 Visualization of molecular docking. The results shown as 3D and 2D diagrams. (**A**) IL6-beta-sitosterol; (**B**) IL6-Stigmasterol; (**C**) ESR1-beta-sitosterol; (**D**) ESR1-beta-sitosterol.

Discussion

Depression is a common complication in CKD patients, seriously affecting the quality of life and survival rate of these patients. Unfortunately, the treatment of CKD patients with western antidepressants has been controversial, and their use has been restricted in clinical practice. Furthermore, most CKD patients do not achieve standardized and good results with this treatment. Studies have confirmed that DSS is effective in treating depression.[7,8] MDSS is a further modified concoction based on DSS to treat depression, which nourishes the kidney, thus benefiting it; however, its specific clinical efficacy and detailed mechanism of action remain unclear.

In the clinical part of this study, compound herbs were used for the first time in the treatment of depression in patients with CKD. As with DSS for depression in general patients,[7,24] MDSS was further optimized with the addition of drugs beneficial to the kidneys and showed significant efficacy in treating depression in patients with CKD. At effective therapeutic doses, MDSS does not cause further development of the renal function, the elevation of hepatic transaminases, or the development of hyperkalemia. In addition, the regimen nourishes the kidneys, tonifies the liver, de-stresses the liver, and calms the mind, effectively improving depressive symptoms and reducing patient suffering. The clinical efficacy and safety of the drug were initially clarified.

We have conducted network pharmacology research on MDSS using network pharmacology, including identification of drug active compounds, key targets of drug effects, signal pathways and molecular docking tests. β-sitosterol, stigmasterol, kaempferol, luteolin, myricanone, and sitosterol were key compounds in MDSS for the treatment of depression in CKD. The pathogenesis of both CKD and depression involves a combination of oxidative stress and inflammatory responses.[25–27] β-sitosterol may regulate the GSH redox cycle by blocking intracellular reactive oxygen species (ROS) accumulation.[28] Furthermore, it had a stronger affinity for estrogen receptors, acting as an antioxidant with the help of estrogen or stimulating antioxidant enzymes, thereby enhancing the protective effect of intracellular antioxidant defense.[29] Moreover, β-sitosterol could inhibit the phosphorylation and degradation of nuclear factor (NF) kappa B (IκB) inhibitors, inhibiting the phosphorylation of NF-κB and extracellular signal-regulated kinase (ERK), thus reducing the expression of inflammatory factors such as inducible nitric oxide (iNOS), tumor necrosis factor (TNF)-α, and cyclooxygenase (COX)-2.[30] Stigmasterol had also been shown to have similar effects in reducing oxidative stress and inflammatory responses. Kaempferol exerted anti-inflammatory activity in vitro and in vivo, inhibiting lipopolysaccharide (LPS)-stimulated production of nitric oxide (NO) and TNF-α[31] and LPS-induced phosphorylation of phosphatidylinositol 3-kinase (PI3K) and serine/threonine kinase (Akt) in cells, thereby reducing cellular inflammatory damage.[32] Luteolin had biological properties such as antioxidant, anti-apoptotic, and anti-inflammatory activities[33] and had been reported to treat chronic inflammatory diseases, acute kidney injury, and neurological disorders, and cancer.[34,35] Furthermore, myricanone had been shown to have anti-inflammatory and antioxidant mechanisms of action.[36] From the results, it was evident that multiple active compounds in MDSS work together to exert multiple synergistic effects, such as anti-inflammatory, antioxidant, and anti-apoptotic effects, which constitute the pharmacological basis of MDSS in the treatment of CKD combined with depression.

Through PPI network analysis, IL6, ESR1, HSP90A1, CASP3, EGFR, PPARG, CCND1, RELA, MDM2, and CASP8 were identified as key targets, which were mainly associated with inflammation, oxidative stress, and apoptosis, which are consistent with the disease characteristics and pathogenesis of CKD and depression.[37,38] To further reveal the possible molecular mechanisms of MDSS for the treatment of CKD combined with depression, we investigated four key targets (IL6, ESR1, HSP90AA1, CASP3) and conducted the corresponding molecular docking with the active compounds. The results showed that the main active compounds of MDSS bound well to all the four key targets, with the strongest binding to IL6 and CASP3. IL6 is an important mediator of inflammatory and immune responses, and its levels are significantly elevated in depressed patients.[39] Furthermore, genetic functional polymorphisms of IL6 influence the severity of depressive symptoms,[40] and pro-inflammatory factors, including IL6, are also strongly associated with the development of kidney-related diseases such as CKD.[41,42] CASP3, a member of the aspartate-specific cysteine protease family, can also be involved in the regulation of depression and kidney-related diseases through immune inflammation and apoptosis pathways.[43,44] These targets thus need to be focused upon for therapeutic purposes.

GO and KEGG analysis revealed that PI3K-Akt signaling pathway, neuroactive ligand-receptor interaction, TNF signaling pathway and apoptotic pathway were the most important pathways associated with MDSS for depression in CKD, in combination with those associated with human disease. Among these signaling pathways, PI3K-Akt signaling pathway was enriched with regard to 14 genes. PI3K-Akt signaling pathway is an important intracellular immune and inflammatory signaling pathway, with the key proteins as PI3K and Akt. Akt not only regulates TNFα and peroxisome proliferator-activated receptor delta (PPARβ/δ) by activating NF-κB but also promotes phosphorylation of murine double minute (MDM)2 to induce degradation of the apoptotic factor p53 and phosphorylates the GSK3 heterodimer at the highly conserved N-terminal regulatory site, thereby regulating GSK3-related apoptosis and glucose metabolism.[45] The above-mentioned anti-inflammatory and antioxidant effects of β-sitosterol are also accomplished through this pathway. In addition, the enrichment of neuroactive ligand-receptor interaction pathway, which is relevant to MDSS for depression, is associated with neuronal differentiation effects such as hippocampal glial cell activation and glioma[46–48] and can synergistically inhibit inflammatory responses with PI3K-Akt signaling pathway.[49] Furthermore, the enrichment of inflammation-associated TNF signaling pathway and apoptotic pathway demonstrated that MDSS treatment of CKD combined with depression involves multiple pathways, multiple targets, and signaling networks of inflammation, oxidative stress, and apoptosis.

Limitations of this trial include the lack of a positive treatment group, the small number of participants, and the short follow-up period. Multicenter clinical trials with longer treatment periods and comparisons with positive treatment groups would be suitable for future studies. It is worth mentioning that this trial was the first to study compound herbal medicine for depression in patients with CKD. Therefore, we recruited only a subset of CKD patients with relatively strong adherence and general condition. Nevertheless, the results of this study emphasize the certain efficacy of MDSS in treating depression in CKD patients. The low impact on renal function and fewer side effects of MDSS compared to classical antidepressants confirm the potential of MDSS as an alternative therapy for depression in CKD patients. On the other hand, this study used a network pharmacology approach to conduct a preliminary exploration of the molecular mechanisms and targets of MDSS action on depression in CKD patients, however, we have not performed animal experimental validation in this study yet due to the complexity of modeling animal models of depression and the subjective aspects of disease assessment. The present results will provide us with a head start for the basic research part of the pharmacologic mechanism to be performed in depth in the near future.

Conclusion

This study showed that treatment with MDSS for 6 weeks improved depressive symptoms in patients with CKD. The key active ingredients of MDSS analyzed by network pharmacology were β-sitosterol, stigmasterol, kaempferol, luteolin, and myricanone, which may act on key targets such as IL6, ESR1, HSP90AA1 and CASP3. The pharmacological mechanism may be mainly related to oxidative stress, inflammation regulation and apoptosis.

Abbreviations

ALT, Alanine aminotransferase; AST, Aminotransferase; BP, Biological Processes; BUN, blood urea nitrogen; CC, Cellular Component; CKD, Chronic kidney disease; CR, Creatinine; COX, Cyclooxygenase; DL, Drug-likeness; DSS, Danggui-Shaoyao-San; ERK, Extracellular signal-regulated kinase; GO, Gene Ontology; HAM-D, Hamilton Depression; iNOS, Inducible nitric oxide; IκB, kappa B; KEGG, Kyoto Encyclopedia of Genes and Genomes; K+, Potassium; LPS, Lipopolysaccharide; MDM, Murine double minute; MDSS, Modified Danggui-Shaoyao-San; MF, Molecular Function; NF, Nuclear factor; OB, Oral bioavailability; NO, Nitric oxide; PI3K, Phosphatidylinositol 3-kinase; PPARβ/δ, Peroxisome proliferator-activated receptor delta; PPI, protein–protein interaction; SD, Standard deviations; ROS, Reactive oxygen species; TNF, Tumor necrosis factor.

Data Sharing Statement

The clinical raw data in this paper will not be shared due to the multicenter clinical studies are still underway. All the data on network pharmacology and molecular docking are available for one year after publication upon request through an e-mail: mengdix@163.com.

Statement of Ethics Approval

This study was approved by the Second Clinical Medical Institution of North Sichuan Medical College (Grant: 2020-134) and directed steadily with the Declaration of Helsinki and the resulting amendments. The preliminary was enrolled at the Chinese clinical preliminary library (www.chictr.org.cn; enlistment number: ChiCTR2100041867).

Consent to Participate Statement

Written informed consent was obtained from all patients prior to their admission, except for three admitted patients over 60 years of age had their family members sign the informed consent on their behalf as inability to write. Consent signed by all patients and potentially their legitimately members were equally valid. Patients were educated that they were allowed to pull out from the preliminary whenever with no unfavorable impact on their relationship with the supplier and their treatment.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Disclosure

The authors have no conflicts of interest to declare.

References

1. Yap SY, Foo CN, Lim YM, et al. Traditional Chinese medicine body constitutions and psychological determinants of depression among university students in Malaysia: a pilot study. *Int J Environ Res Public Health*. 2021;18(10). doi:10.3390/ijerph18105366
2. Preljevic VT, Østhus TB, Os I, et al. Anxiety and depressive disorders in dialysis patients: association to health-related quality of life and mortality. *Gen Hosp Psychiatry*. 2013;35(6):619–624. doi:10.1016/j.genhosppsych.2013.05.006
3. Boulware LE, Liu Y, Fink NE, et al. Temporal relation among depression symptoms, cardiovascular disease events, and mortality in end-stage renal disease: contribution of reverse causality. *Clin J Am Soc Nephrol*. 2006;1(3):496–504. doi:10.2215/CJN.00030505
4. Hedayati SS, Minhajuddin AT, Afshar M, Toto RD, Trivedi MH, Rush AJ. Association between major depressive episodes in patients with chronic kidney disease and initiation of dialysis, hospitalization, or death. *JAMA*. 2010;303(19):1946–1953. doi:10.1001/jama.2010.619
5. Cohen LM, Tessier EG, Germain MJ, Levy NB. Update on psychotropic medication use in renal disease. *Psychosomatics*. 2004;45(1):34–48. doi:10.1176/appi.psy.45.1.34
6. Hedayati SS, Bosworth HB, Kuchibhatla M, Kimmel PL, Szczech LA. The predictive value of self-report scales compared with physician diagnosis of depression in hemodialysis patients. *Kidney Int*. 2006;69(9):1662–1668. doi:10.1038/sj.ki.5000308
7. Li H, Li D, Zhao G, Gao Y, Ke J. Effects of Danggui-Shaoyao-San on depression- and anxiety-like behaviors of rats induced by experimental tooth movement. *J Orofac Orthop*. 2022;83(1):23–33. doi:10.1007/s00056-021-00323-0
8. Xu F, Peng D, Tao C, et al. Anti-depression effects of Danggui-Shaoyao-San, a fixed combination of Traditional Chinese Medicine, on depression model in mice and rats. *Phytomedicine*. 2011;18(13):1130–1136. doi:10.1016/j.phymed.2011.05.002
9. American Psychiatric Association (APA). *Diagnostic and Statistical Manual of Mental Disorders (DSM-5)*. 5th ed. Washington, DC: American Psychiatric Association; 2013.
10. Zhong J, Yang HC, Fogo AB. A perspective on chronic kidney disease progression. *Am J Physiol Renal Physiol*. 2017;312(3):F375–F384. doi:10.1152/ajprenal.00266.2016
11. Medicine CAoC. Guidelines for the treatment of common diseases in internal medicine. In: *Medical Conditions Section*. Beijing: China Press of Traditional Chinese Medicine; 2008:294–296.
12. Pan S, Liu ZW, Shi S, et al. Hamilton rating scale for depression-24 (HAM-D(24)) as a novel predictor for diabetic microvascular complications in type 2 diabetes mellitus patients. *Psychiatry Res*. 2017;258:177–183. doi:10.1016/j.psychres.2017.07.050

13. Kashani L, Eslatmanesh S, Saedi N, et al. Comparison of saffron versus fluoxetine in treatment of mild to moderate postpartum depression: a double-blind, randomized clinical trial. *Pharmacopsychiatry*. 2017;50(2):64–68. doi:10.1055/s-0042-115306
14. Ru J, Li P, Wang J, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. *J Cheminform*. 2014;6:13. doi:10.1186/1758-2946-6-13
15. Zhang W, Chao X, Wu JQ, et al. Exploring the potential mechanism of Guchang Zhixie Wan for treating ulcerative colitis by comprehensive network pharmacological approaches and molecular docking validation as well as cell experiments. *Chem Biodivers*. 2021;18(1):e2000810. doi:10.1002/cbdv.202000810
16. Davis AP, Grondin CJ, Johnson RJ, et al. Comparative Toxicogenomics Database (CTD): update 2021. *Nucleic Acids Res*. 2021;49(D1):D1138–D1143. doi:10.1093/nar/gkaa891
17. Stelzer G, Dalah I, Stein TI, et al. In-silico human genomics with GeneCards. *Hum Genomics*. 2011;5(6):709–717. doi:10.1186/1479-7364-5-6-709
18. Amberger JS, Hamosh A. Searching Online Mendelian Inheritance in Man (OMIM): a knowledgebase of human genes and genetic phenotypes. *Curr Protoc Bioinformatics*. 2017;58:1.2.1–2.12. doi:10.1002/cpbi.27
19. Piñero J, Bravo À, Queralt-Rosinach N, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants. *Nucleic Acids Res*. 2017;45(D1):D833–d9. doi:10.1093/nar/gkw943
20. von Mering C, Jensen LJ, Snel B, et al. STRING: known and predicted protein-protein associations, integrated and transferred across organisms. *Nucleic Acids Res*. 2005;33:D433–D437. doi:10.1093/nar/gki005
21. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat Commun*. 2019;10(1):1523. doi:10.1038/s41467-019-09234-6
22. Burley SK, Berman HM, Christie C, et al. RCSB Protein Data Bank: sustaining a living digital data resource that enables breakthroughs in scientific research and biomedical education. *Protein Sci*. 2018;27(1):316–330. doi:10.1002/pro.3331
23. Kim S, Chen J, Cheng T, et al. PubChem in 2021: new data content and improved web interfaces. *Nucleic Acids Res*. 2021;49(D1):D1388–D1395. doi:10.1093/nar/gkaa971
24. Huang Z, Mao QQ, Zhong XM, Li ZY, Qiu FM, Ip SP. Mechanistic study on the antidepressant-like effect of danggui-shaoyao-san, a Chinese herbal formula. *Evid Based Complement Alternat Med*. 2012;2012:173565. doi:10.1155/2012/173565
25. Rapa SF, Di Iorio BR, Campiglia P, Heidland A, Marzocco S. Inflammation and oxidative stress in chronic kidney disease-potential therapeutic role of minerals, vitamins and plant-derived metabolites. *Int J Mol Sci*. 2019;21(1):263. doi:10.3390/ijms21010263
26. Gałecki P, Talarowska M. Inflammatory theory of depression. *Psychiatr Pol*. 2018;52(3):437–447. doi:10.12740/PP/76863
27. Lindqvist D, Dhabhar FS, James SJ, et al. Oxidative stress, inflammation and treatment response in major depression. *Psychoneuroendocrinology*. 2017;76:197–205. doi:10.1016/j.psyneuen.2016.11.031
28. Li JL, Okada S, Hamazaki S, Ebina Y, Midorikawa O. Subacute nephrotoxicity and induction of renal cell carcinoma in mice treated with ferric nitrilotriacetate. *Cancer Res*. 1987;47(7):1867–1869.
29. Strehlow K, Rotter S, Wassmann S, et al. Modulation of antioxidant enzyme expression and function by estrogen. *Circ Res*. 2003;93(2):170–177. doi:10.1161/01.RES.0000082334.17947.11
30. Sun Y, Gao L, Hou W, Wu J. β-sitosterol alleviates inflammatory response via inhibiting the activation of ERK/p38 and NF-κB pathways in LPS-exposed BV2 cells. *Biomed Res Int*. 2020;2020:7532306. doi:10.1155/2020/7532306
31. Tran MH, Nguyen HD, Kim JC, Choi JS, Lee HK, Min BS. Phenolic glycosides from Alangium salviifolium leaves with inhibitory activity on LPS-induced NO, PGE(2), and TNF-alpha production. *Bioorg Med Chem Lett*. 2009;19(15):4389–4393. doi:10.1016/j.bmcl.2009.05.070
32. Park SE, Sapkota K, Kim S, Kim H, Kim SJ. Kaempferol acts through mitogen-activated protein kinases and protein kinase B/AKT to elicit protection in a model of neuroinflammation in BV2 microglial cells. *Br J Pharmacol*. 2011;164(3):1008–1025. doi:10.1111/j.1476-5381.2011.01389.x
33. Cordaro M, Cuzzocrea S, Crupi R. An update of palmitoylethanolamide and luteolin effects in preclinical and clinical studies of neuroinflammatory events. *Antioxidants*. 2020;9(3):216. doi:10.3390/antiox9030216
34. Hong X, Zhao X, Wang G, Zhang Z, Pei H, Liu Z. Luteolin treatment protects against renal ischemia-reperfusion injury in rats. *Mediators Inflamm*. 2017;2017:9783893. doi:10.1155/2017/9783893
35. Liu YS, Yang Q, Li S, et al. Luteolin attenuates angiotensin II-induced renal damage in apolipoprotein E-deficient mice. *Mol Med Rep*. 2021;23(2). doi:10.3892/mmr.2020.11796
36. R M Ibrahim S, A Mohamed G, I M Khedr A, M Aljaeid B. Anti-oxidant and anti-inflammatory cyclic diarylheptanoids from alnus japonica stem bark. *Iran J Pharm Res*. 2017;16(Suppl):83–91.
37. Trzonkowski P, Myśliwska J, Godlewska B, et al. Immune consequences of the spontaneous pro-inflammatory status in depressed elderly patients. *Brain Behav Immun*. 2004;18(2):135–148. doi:10.1016/S0889-1591(03)00111-9
38. Maksimowski N, Williams VR, Scholey JW, Shimosawa T. Kidney ACE2 expression: implications for chronic kidney disease. *PLoS One*. 2020;15(10):e0241534. doi:10.1371/journal.pone.0241534
39. Ryan J, Pilkington L, Neuhaus K, Ritchie K, Ancelin ML, Saffery R. Investigating the epigenetic profile of the inflammatory gene IL-6 in late-life depression. *BMC Psychiatry*. 2017;17(1):354. doi:10.1186/s12888-017-1515-8
40. Frydecka D, Pawłowski T, Pawlak D, Małyszczak K. Functional polymorphism in the interleukin 6 (IL6) gene with respect to depression induced in the course of interferon-α and ribavirin treatment in chronic hepatitis patients. *Arch Immunol Ther Exp*. 2016;64(Suppl 1):169–175. doi:10.1007/s00005-016-0441-7
41. Okada R, Wakai K, Naito M, et al. Pro-/anti-inflammatory cytokine gene polymorphisms and chronic kidney disease: a cross-sectional study. *BMC Nephrol*. 2012;13:2. doi:10.1186/1471-2369-13-2
42. Verzola D, Cappuccino L, D'Amato E, et al. Enhanced glomerular Toll-like receptor 4 expression and signaling in patients with type 2 diabetic nephropathy and microalbuminuria. *Kidney Int*. 2014;86(6):1229–1243. doi:10.1038/ki.2014.116
43. Lan S, Yang B, Migneault F, et al. Caspase-3-dependent peritubular capillary dysfunction is pivotal for the transition from acute to chronic kidney disease after acute ischemia-reperfusion injury. *Am J Physiol Renal Physiol*. 2021;321(3):F335–F351. doi:10.1152/ajprenal.00690.2020
44. Han MH, Jiao S, Jia JM, et al. The novel caspase-3 substrate Gap43 is involved in AMPA receptor endocytosis and long-term depression. *Mol Cell Proteomics*. 2013;12(12):3719–3731. doi:10.1074/mcp.M113.030676

45. Wang K, Scheel-Toellner D, Wong SH, et al. Inhibition of neutrophil apoptosis by type 1 IFN depends on cross-talk between phosphoinositol 3-kinase, protein kinase C-delta, and NF-kappa B signaling pathways. *J Immunol*. 2003;171(2):1035–1041. doi:10.4049/jimmunol.171.2.1035
46. Chen J, Wang Z, Wang W, et al. SYT16 is a prognostic biomarker and correlated with immune infiltrates in glioma: a study based on TCGA data. *Int Immunopharmacol*. 2020;84:106490. doi:10.1016/j.intimp.2020.106490
47. Wang J, Yue B, Zhang X, Guo X, Sun Z, Niu R. Effect of exercise on microglial activation and transcriptome of hippocampus in fluorosis mice. *Sci Total Environ*. 2021;760:143376. doi:10.1016/j.scitotenv.2020.143376
48. Zhou L, Tang H, Wang F, et al. Bioinformatics analyses of significant genes, related pathways and candidate prognostic biomarkers in glioblastoma. *Mol Med Rep*. 2018;18(5):4185–4196. doi:10.3892/mmr.2018.9411
49. He J, Zhang N, Zhu Y, Jin R, Wu F. MSC spheroids-loaded collagen hydrogels simultaneously promote neuronal differentiation and suppress inflammatory reaction through PI3K-Akt signaling pathway. *Biomaterials*. 2021;265:120448. doi:10.1016/j.biomaterials.2020.120448