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Study on Mechanism of Huangqi-Shanyao-Danshen Compatibility in Treatment of Diabetic Nephropathy Based on GEO Database and Network Pharmacology and Molecular Docking

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Abstract: To explore the significance of Huangqi in the treatment of DN, this article explored the potential targets and signaling pathways of Astragalus-Chinese yam-Salvia miltiorrhiza in the intervention of diabetic nephropathy (DN), based on GEO database combined with network pharmacology and molecular docking. The relevant chips of DN were searched by GEO database, and the chemical active substances and corresponding targets in Astragali Radix, Dioscoreae Rhizoma and Salviae Miltiorrhizae Radix et Rhizoma were screened by TCMSP database, and the intersection with differentially expressed genes was taken. Then, the PPI network diagram was constructed by String database and Cytoscape 3.9.0 software respectively, and the gene GO and KEGG enrichment analysis were carried out by R language, and the above research results were verified by molecular docking. The results of molecular docking showed that the receptors IL6 and IL1 B could stably bind and interact with quercetin, diosgenin and luteolin. Thus, the active components such as quercetin, luteolin and diosgenin of Astragalus-Dioscorea-Salvia miltiorrhiza play a therapeutic role in DN by acting on IL6, IL1 B, MM9 and other targets, regulating AGE-RAGE and other signaling pathways.

1. Introduction

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease worldwide [1]. At present, the treatment is mainly based on general treatment and lifestyle changes [2]. Due to the limited treatment methods of western medicine, there are many shortcomings. The advantages of traditional Chinese medicine in the treatment of DN are gradually rising. By analyzing the etiology and pathogenesis of DN, traditional Chinese medicine determines the deficiency of the root and the excess of the branch. The combination of disease differentiation and syndrome differentiation, the use of drugs in stages, and the control of renal function deterioration have achieved good results [3].

According to the literature mining, the commonly used Chinese medicine for the treatment of DN is Huangqi-Shanyao-Danshen [4]. Huangqi is a key medicine for tonifying middle qi and replenishing qi. Huangqi can improve the early renal damage of patients with type 2 diabetes with qi deficiency syndrome, reduce the levels of urinary L-FABP and ACR, improve the symptoms of qi deficiency, and prevent DN [5]. Chinese yam has the effect of tonifying kidney and consolidating essence, tonifying qi and nourishing yin, and tonifying spleen and lung. Through experiments, it is pointed out that the component yam polysaccharide can reduce the weight of DN rats and improve renal function [6]; salvia miltiorrhiza has the effect of promoting blood circulation and removing blood stasis. Salvia miltiorrhiza has the effect of reducing blood glucose in the treatment of DN, so it can prevent the progress of DN [7]. In this study, the differential genes of DN were mined from GEO database combined with network pharmacology to predict the active components and molecular mechanisms of Huangqi-Shanyao-Danshen compatibility in the treatment of DN, and further verified by molecular docking, so as to provide scientific basis for the experimental research and clinical application of Huangqi-Shanyao-Danshen compatibility in the treatment of DN.

2. Materials and Methods

2.1. GEO analysis of differential genes in DN

The relevant data of DN were searched through the GEO database of NCBI, and the gene chip GSE142153 containing DN data was selected. The platform was GPL6480. The original data of the chip included 10 normal human kidney samples and 23 DN samples. R language was used to analyze the differential genes in the gene chip, and the screening conditions were: Pvalue < 0.05.

2.2. Screening of active components and potential targets of Astragalus membranaceus-Yam-Salvia miltiorrhiza

The active ingredients of Radix Astragali, Rhizoma Dioscoreae and Radix Salviae Miltiorrhizae were obtained through TCMSP database (https://old.tcmsp-e.com/tcmsp.php),). The oral bioavailability (OB) ≥ 30 % and drug-likeness (DL) ≥ 0.18 were used as the criteria to screen out the active ingredients and related targets, and then the Perl language was used to standardize the name of the target.

2.3. Analysis of active components and key targets of Huangqi-Shanyao-Danshen in the treatment of DN

Cytoscape 3.9.0 was used to draw the active ingredient-target network diagram of Huangqi-Shanyao-Danshen compatibility in the treatment of DN, and then PPI analysis was performed through the STRING database. After network analysis, the results of interaction analysis between genes were obtained. The file was imported into Cytoscape 3.9.0 software to draw the pathway-target network diagram. R language is used for topology analysis and verification.

2.4. Enrichment analysis of intersection targets

The script was set according to the intersection target data and imported into the R language. The correction p value was set to 0.05, and the GO enrichment analysis and KEGG enrichment analysis were performed. The first ten items were set to display only. Finally, the results of the key target-signaling pathway were output by the mapping software Cytoscape 3.9.0.

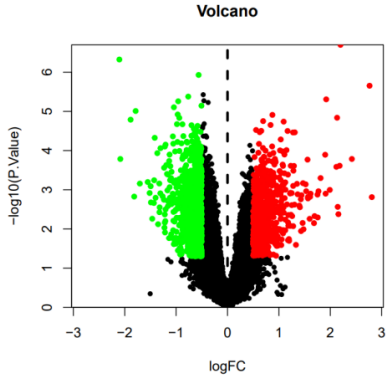
2.5. Molecular docking verification of active components of drugs and key targets

The top three compounds of the above drugs were obtained by using the PubChem database to obtain the molecular structure, that is, the ligand small molecule, and the 2D structure of the small molecule was drawn with ChemDraw20.0, and then substituted into Chem3D 20.0 to draw the 3D structure, and the RCSB PDB database was used to obtain the receptor. Autodock vina software was used for molecular docking of ligands and receptors, and the two groups with the most stable binding force were visualized by PyMol software.

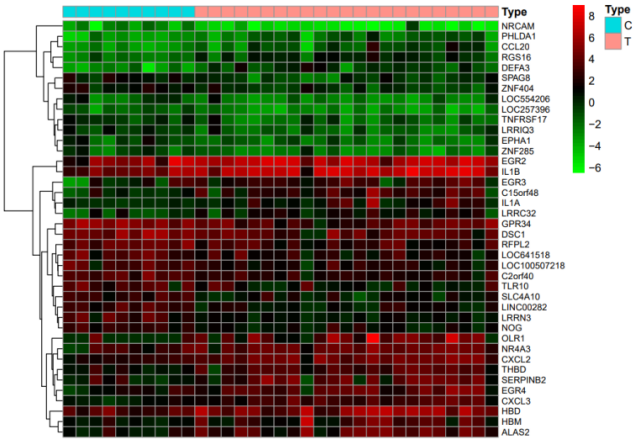
3. Results

3.1. Analysis of differentially expressed genes in DN

In the chip GSE142153, 10 normal human kidney samples were set as the normal group, and 23 DN samples were set as the experimental group. Through R language analysis, a total of 1390 differentially expressed genes were obtained. In the experimental group, 661 genes were up-regulated and 729 genes were down-regulated. The results are shown in Figure 1A volcano map. Differential gene enrichment analysis was performed on the 20 genes with the most significant up-regulation and down-regulation, and the results were shown in Figure 1B.



A: Black represents no differential genes, green represents up-regulated genes in the normal group, and red represents up-regulated genes in the experimental group.



B: The abscissa represents the sample name, the ordinate represents the gene name, the normal group sample is in the front, the DN group sample is in the back, the green represents the low expression in the sample, the black represents the expression, and the red represents the high expression.

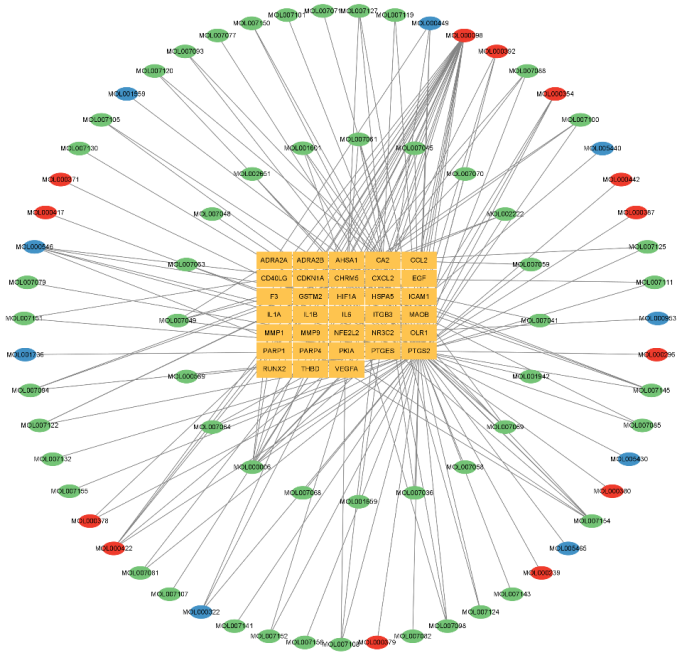
Figure 1: Differentially expressed genes and enrichment results

3.2. Screening of active compounds and corresponding targets of Huangqi-Shanyao-Danshen compatibility

Through TCMSP query, after screening by OB ≥ 30 and DL ≥ 0.18, the effective chemical components of Astragalus, Chinese yam and Salvia miltiorrhiza were 20, 16 and 65, respectively. Through database analysis, the targets of Astragali Radix, Dioscoreae Rhizoma and Salviae Miltiorrhizae Radix et Rhizoma were 405,122 and 883, respectively. A total of 33 intersection genes of active components and diseases were obtained. They were CHRM5, PTGS2, CA2, NR3C2, VEGFA, CDKN1A, MMP9, IL6, MMP1, ICAM1, CD40LG, PTGES, ADRA2A, ADRA2B, AHSA1, PARP4, ITGB3, MAOB, OLR1, PKIA, GSTM2, EGF, HIF1A, HSPA5, F3, IL1B, CCL2, THBD, IL1A, NFE2L2, PARP1, CXCL2, RUNX2.

3.3. Analysis of active components and key targets of Huangqi-Shanyao-Danshen in the treatment of DN

Cytoscape 3.9.0 was used to draw the active ingredient-target network diagram of the three drugs in the treatment of DN, and the results are shown in figure 2. Quercetin (MOL000098), diosgenin (MOL000546) and luteolin (MOL000006) were the top compounds in the order of value. The String platform and TSV file were used to import Cytoscape 3.9.0 to draw PPI network diagram (Figure 3), and the top ten degree values were obtained as important targets for drug treatment of DN.Then R language was used for topology analysis and verification (Figure 4). Finally, the top ten targets of degree values were IL6, IL1 B, MMP9, VEGFA, PTGS2, ICAM1, EGF, CCL2, HIF1 A and F3, respectively. It is speculated that it may be the key target of Astragalus-Yam-Salvia miltiorrhiza drug pair in the treatment of DN.



Note: Green, red, yellow, and blue nodes represent the active components of Salvia miltiorrhiza, the active components of Astragalus membranaceus, the intersection targets, and yam, respectively.

Figure 2: Active ingredient-target network diagram of drug treatment for diseases

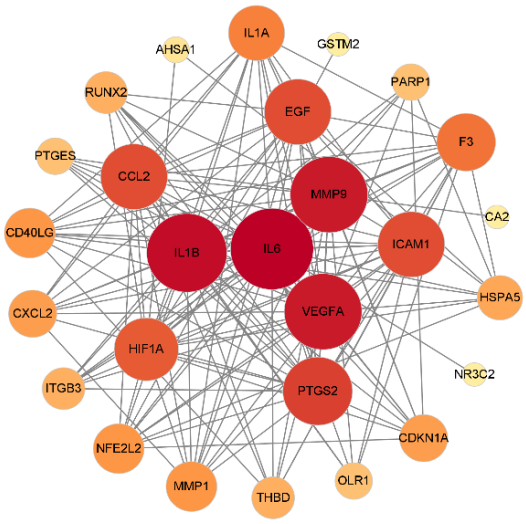


Figure 3: PPI network diagram of drug-disease-related target intersection genes

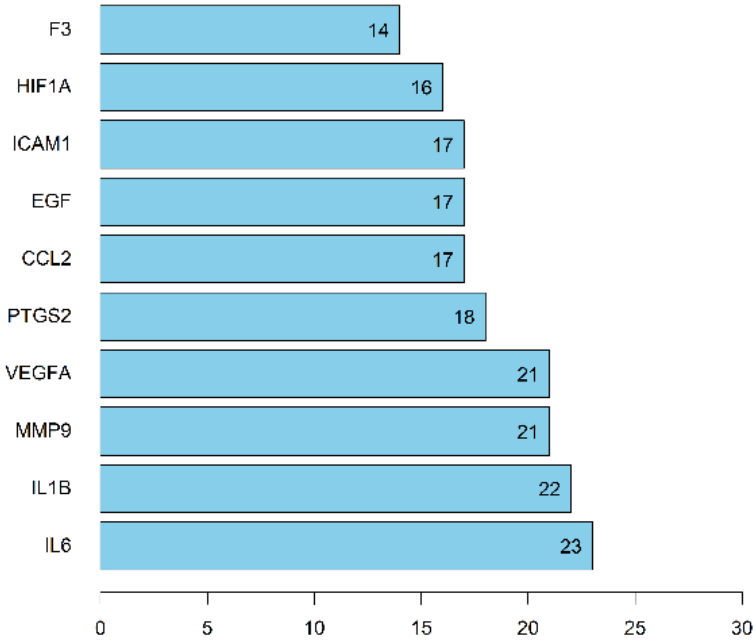


Figure 4: Topological properties of core targets of drug intervention in diseases

3.4. Enrichment analysis

3.4.1. GO enrichment analysis

R language was used to analyze the GO functional enrichment of 33 core targets involved in Huangqi-Shanyao-Danshen-DN. The longer the column and the redder the color, the higher the significance of enrichment. The results of biological process enrichment showed that wound healing, regulation of body fluid levels and acute inflammatory response were important biological processes (see Fig. 5).

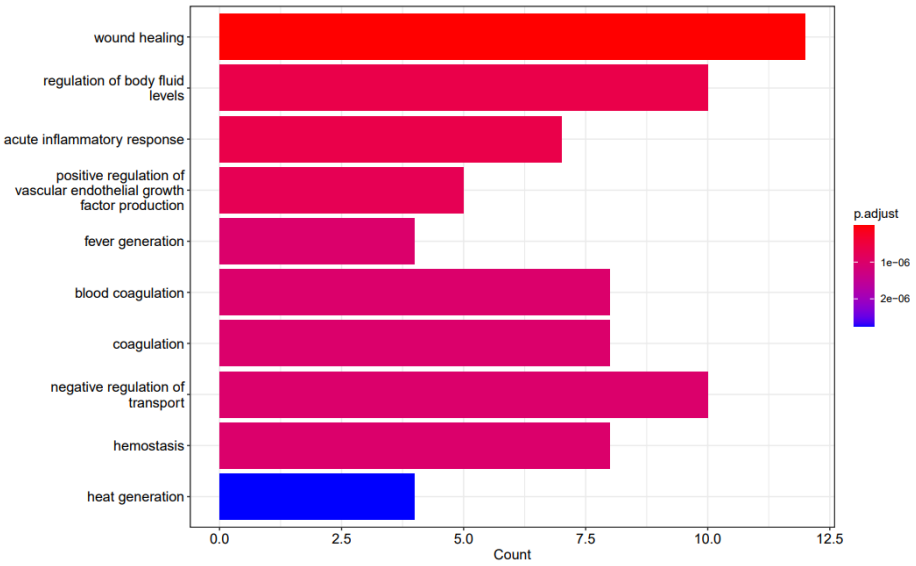


Figure 5: GO functional enrichment analysis of biological process histogram

3.4.2. KEGG pathway enrichment analysis

KEGG pathway enrichment analysis obtained 42 signal pathways, and R language was used to visualize the top ten. The key targets of drug treatment for DN were mainly enriched in the AGE-RAGE signaling pathway in fluid shear stress and atherosclerosis, lipid and atherosclerosis, and diabetic complications, as shown in Fig. 6. The relationship between KEGG pathway and target was visualized by Cytoscape 3.9.0, and analyzed from the perspective of target. It can be seen that the more important protein targets are IL1B, ICAM1, and IL6 regulating hsa05417, hsa05418, and hsa04933 pathways, as shown in Figure 7.

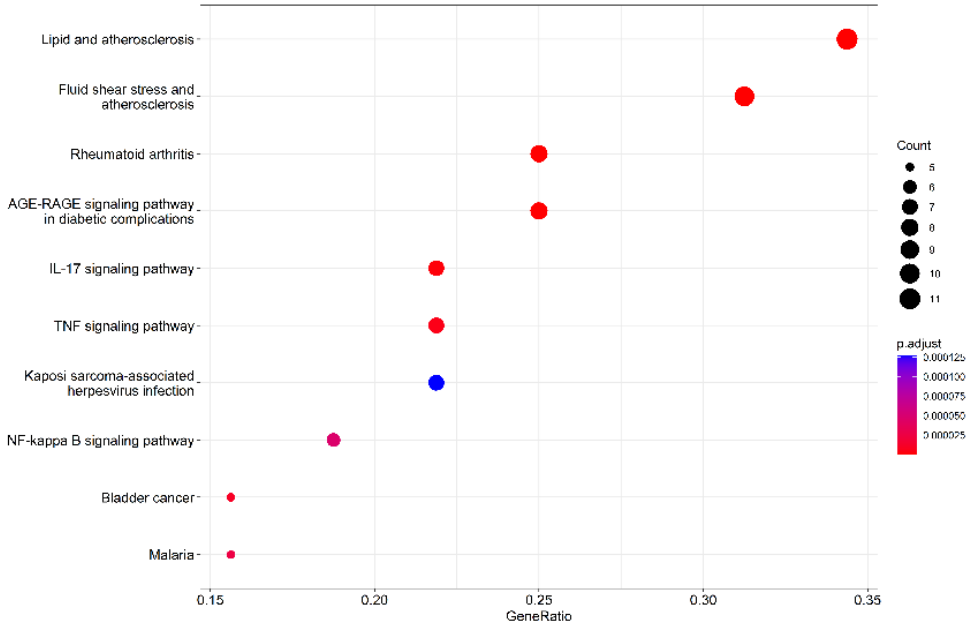


Figure 6: Bubble diagram of KEGG enrichment analysis of the main target of Astragalus-Yam-Salvia miltiorrhiza

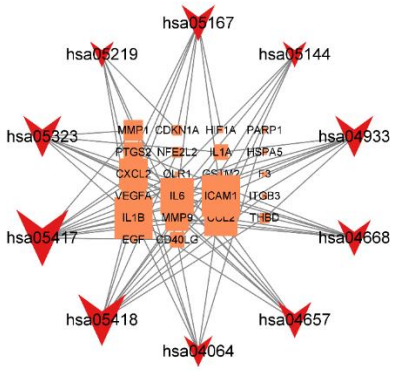


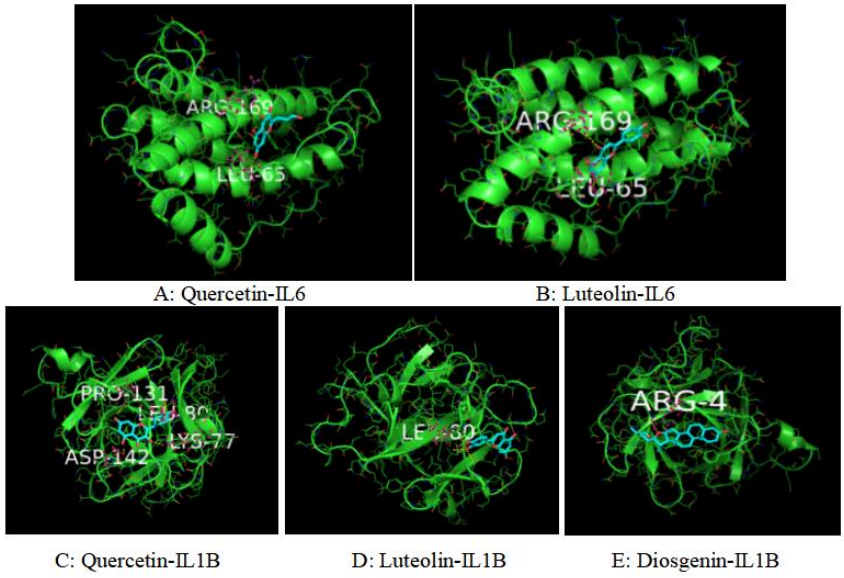
Figure 7: KEGG pathway-target network diagram

3.5. Molecular docking results

Based on the above results, molecular docking was performed using the top three quercetin, diosgenin, luteolin and the top two core targets IL6 and IL1 B of PPI, respectively. The best binding energy results are shown in Table 1, and the binding force is shown in Figure 8. The results showed that the binding energies of IL6 and IL1 B with quercetin, diosgenin and luteolin were-6.4 kcal ·mol-1, indicating that the two receptors could bind and interact stably.

Table 1: Molecular docking results of active components and core targets

active compounds	binding energy (kcal/mol)	
	IL1B	IL6
quercetin	-6.5	-6.8
luteolin	-6.5	-7.5
Diosgenin	-7.3	-8.7



A: Quercetin-IL6 B: Luteolin-IL6

C: Quercetin-IL1B D: Luteolin-IL1B E: Diosgenin-IL1B

Figure 8: The best combination of active ingredients and core targets

4. Discussions

Traditional Chinese medicine believes that DN is based on the deficiency of spleen and kidney qi and yin as the key pathogenesis [8]. The pathogenesis of DN is the consequence of diabetes. Therefore, the pathogenesis of DN must be combined with diabetes. In terms of diabetes, it is divided into upper, middle and lower, but it is all related to the spleen. The upper elimination is caused by the disorder of spleen transportation and transformation, which leads to the inability of the lung to distribute body fluid; zhongxiao is caused by spleen-stomach disharmony; it is caused by the disorder of kidney and spleen; the kidney collaterals stasis exists in the whole process [9], and yam has the effect of tonifying kidney, nourishing qi and yin, tonifying spleen and lung, which can treat DN from the deficiency of spleen and kidney qi and yin. Salvia miltiorrhiza has the effect of promoting blood circulation and removing blood stasis, which can improve DN in the treatment of kidney collaterals stasis.

The rats with diabetic nephropathy were treated with yam powder. The results showed that yam could improve the lesions of liver, kidney and pancreas in diabetic rats to a certain extent, and could reduce blood glucose [10]. Many experiments have proved that astragalus has the effect of treating diabetic nephropathy, such as astragaloside [11, 12], astragalus polysaccharide [13], which can reduce renal tissue damage and delay the progression of renal function. Tanshinone II can reduce insulin-like growth factor 1 and plasma protein C to treat DN [14]. Therefore, this paper studies the treatment of DN with Huangqi-Shanyao-Danshen from the perspective of network pharmacology combined with GEO database.

Quercetin has anti-inflammatory effect [15], quercetin can reduce blood sugar and improve the development of DN [16]; luteolin can activate antioxidant proteins by up-regulating inflammatory factors, delay kidney damage [17], and improve DN. Diosgenin has lipid-lowering, anti-inflammatory and other effects, which can reduce the expression of inflammatory factors (IL-1β, IL-6) [18].

In this study, through GEO data analysis, it was found that IL6, IL1 B, etc.ranked high in the Degree value of PPI network, suggesting that the above targets may be the main targets for DN treatment. Clinical experiments have pointed out that IL-6 mRNA is expressed in renal tubular cells, glomerular mesangial cells and other cells. Increased expression has played a key role in the development of DN [19]. Clinical trials have pointed out that plasma IL-1B level can be used as a detection index for the development of DN patients [20], which is positively correlated with the stage of DN, and belongs to the up-regulated gene of DN group.

The results of GO and KEGG enrichment showed that the signal pathways of fluid shear stress and atherosclerosis, lipid and atherosclerosis were closely related to the pathogenesis of DN. By measuring the serum of DN patients and normal people [21], it was found that the experimental group corresponding to atherosclerosis had higher expression of inflammatory factors, such as up-regulating inflammatory factors such as IL1 B and IL6, which confirmed that atherosclerosis was closely related to DN. AGE-RAGE is an advanced glycation end product. By comparing the renal dysfunction group, the massive proteinuria group and the microalbuminuria group [22], it was found that the serum AGEs level was closely related to the severity of type 2 DN. At the same time, through molecular docking, the results also showed that quercetin, luteolin and diosgenin could be stably docked with key targets IL6 and IL1 B, which played a therapeutic role in DN.

This study explored that quercetin, luteolin and diosgenin in Astragalus-Yam-Salvia miltiorrhiza may regulate fluid shear stress and atherosclerosis, lipid and atherosclerosis by acting on core targets such as IL1B and IL6 to treat DN, and provide more research targets and pathways for the experimental and clinical research of Astragalus-Yam-Salvia miltiorrhiza in the treatment of DN in the future.

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