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Research Article

Study on the Effect of Rhubarb and Its Active Components on Pyroptosis in DKD by Regulating STAT3/Caspase-11 Axis

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Abstract

Rhubarb has been found to have a certain protective effect on improving the kidney function. However, the specific mechanism is still unclear. In this study, network pharmacology, molecular docking spontaneous binding technology and molecular biology experiments were used to verify the mechanism of rhubarb and its active ingredients in the treatment of DKD. A total of 10 active compounds and 121 (larger than average) target proteins were collected. The target proteins with higher degree value were screened by PPI according to degree value as follows: AKT1, STAT3, EGFR, NFKB1, SRC, etc. GO and KEGG enrichment analysis suggest that rhubarb therapy for DKD mainly involves Pathways in cancer, Prostate cancer, Proteoglycans in cancer, Chemokine signaling pathway, PI3K-Akt signaling pathway, PD-L1 expression and PD-1 checkpoint pathway in cancer, EGFR tyrosine kinase inhibitor resistance signaling pathway and so on. Furthermore, molecular docking results suggest that hydrogen bonding, salt bridge and hydrophobic interactions contribute to spontaneous binding of the compound to the target protein. Experimental verification shows that rhubarb and aloe emodin affect the mechanism of pyroptosis in diabetic kidney disease by regulating STAT3/Caspase11 axis. In conclusion, this study comprehensively elaborated the active compounds, potential targets and molecular experimental mechanisms of rhubarb to provide the basic experimental theory for clinical treatment of DKD.

Keywords

Network Pharmacology, Rhubarb, SATA3, Pyroptosis, DKD

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1. Introduction

As early as 1764, Cotunnius reported for the first time that persistent diabetes had a certain impact on the kidney. In 1877, Armanni and Epstein first discovered the specific pathological damage of the kidney under the microscope of DKD. In 1983, Mogensen CE conducted the staging of DKD, which has been used as a reference for clinical diagnosis and treatment. DKD is one of the most common microvascular complications induced by chronic hyperglycemia in diabetes mellitus and has a high disability rate. In recent years, the incidence of diabetes has increased year by year, and the disease has become the main cause of chronic kidney disease and end-stage renal disease. The risk factors of diabetic nephropathy include age, hyperglycemia, hypertension, obesity, hyperlipidemia and so on. Its pathogenesis is complex. Studies have been conducted, including abnormal renal hemodynamics, inflammation, oxidative stress, and disorders of glucose and lipid metabolism, all of which are involved in the occurrence and development of the disease. The pathological manifestations began with the increase of kidney volume at the initial stage, followed by glomerular basement membrane thickening and mesangial matrix increase as the disease progressed, and the disease further progressed to massive fibrinogen deposition, glomerular sclerosis, Kimnel-Steil-Wilson (K-W) nodules formation, and finally irreversible damage. Clinical DKD patients often have entered the stage IV or even the terminal stage of diabetic nephropathy when the disease is found [1-3].

At present, the treatment of DKD in modern medicine mainly includes basic treatment such as controlling blood sugar and blood pressure, regulating lipid metabolism, etc. The lack of effective specific treatment methods has brought huge mental and economic burden to patients and their families, and seriously affected their quality of life. Traditional Chinese medicine (TCM) is a mature traditional medicine that has been continuously fighting against diseases in life practice for thousands of years in China, and has significant advantages in the treatment of DKD [4-6].

According to the main clinical manifestations of DKD, Chinese medicine classified it into the categories of "kidney elimination", "Xiaothirst", "edema" and "turbidity-urine", and emphasized that the disease location was mainly in the kidney, and its pathological nature was the original deficiency, which was the deficiency of Qi and blood, Yin and Yang and the five viscera, and the standard was the deficiency of water dampness, blood stasis and turbidity-phlegm [7-9]. Many ancient books record that rhubarb is the essential medicine for purging and invading accumulation, which was first recorded in Shennong's Herbal Classic. Alias General, Huang Liang, Fire ginseng, etc. Smell bitter cold, non-toxic, the main blood stasis, Blood blocking cold and heat, breaking the accumulation of mass, Drink vegetarian food, cleanse the stomach, Push the old to the new, easing intestines and stomach, Regulating the medium to transform food, Peace and five viscera. The main effects are diarrhea and accumulation, cooling and detoxification, Expelling stasis through menstruation, removing dampness and withdrawing yellow. It is recorded in "Medicine Zhongshen Xilu": "Rhubarb taste bitter, fragrant, cool, can enter the blood, break all blood stasis." Because the pathogenesis of DKD is kidney collateral stasis, and rhubarb has the function of clearing blood stasis and dredging meridians, rhubarb has a better effect on targeted treatment of DKD. At present, there are only four kinds of rhubarb products recorded in the Pharmacopoeia of the People's Republic of China (Part I) (referred to as the "Chinese Pharmacopoeia"), namely, raw rhubarb, cooked rhubarb, wine rhubarb and rhubarb charcoal. Raw rhubarb has strong catharsis; Cooked rhubarb purging slow, can purging fire detoxification; Wine rhubarb good clear upper jiao blood heat poison; Rhubarb charcoal is used to cool blood and remove stasis. Other studies have found that among the four kinds of rhubarb products, wine rhubarb has the strongest effect on promoting blood circulation and removing blood stasis, while cooked rhubarb is slightly weaker. Raw rhubarb has certain effect on promoting blood circulation and removing blood stasis, while charcoal rhubarb has no effect on promoting blood circulation and removing blood stasis [10-13].

Yishentongluo formula is one of the classic Chinese medicine compounds used for DKD treatment, which consists of 7 kinds of medicines: Salvia miltiorrhiza, Astragalus, Fructus Corni, Rhubarb, cooked Rehmannia, dragon's blood, Euonymus alatus. Previous studies by our research group have found that Yishentongluo can improve DKD through multiple signaling pathways and multiple targets, but its mechanism of action has not been fully clarified [14]. Rhubarb is a supplementary drug in Yishentongluo prescription. This study intends to further explore the role and possible mechanism of rhubarb and its active components in DKD through network pharmacology and molecular experiments on the basis of our previous research group. Working flow chart of rhubarb for DKD (Figure 1).

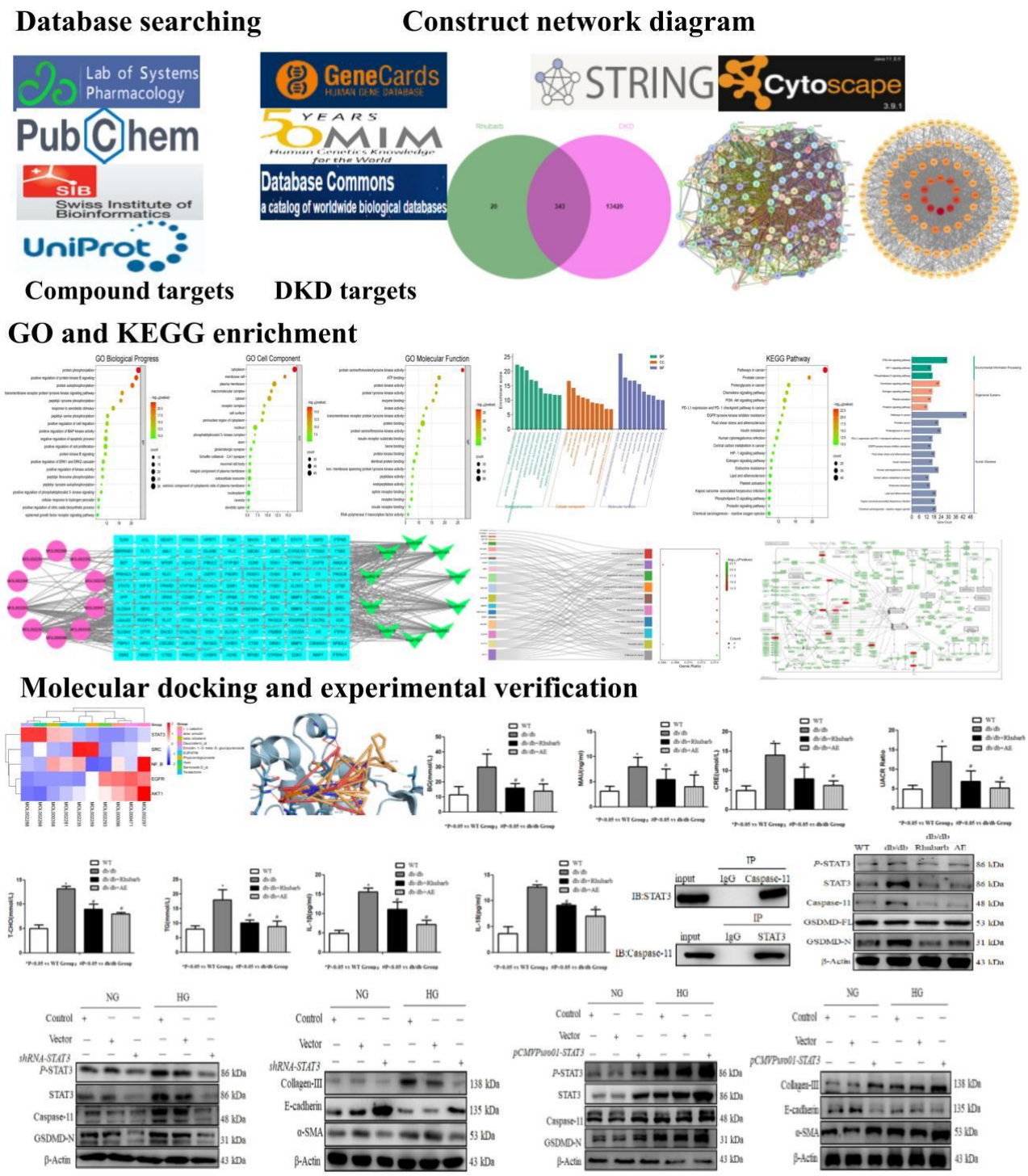

Figure 1. *To study the working flow of rhubarb intervention mechanism for DKD.*

2. Materials and Methods

2.1. Animal Experiment Verification

Experimental animals: clean Wild type (WT) control mouse, male, body weight (20±5); db/db mouse, male, weight (40±10)g. Purchased from Jiangsu Zhichaokang Biotechnology Co, LTD, Lot number: [SCXK(Su)2018-0008].

2.2. Cell Transfection

Mouse renal tubular epithelial cells (mRTEC) cells were purchased from Guangzhou Genio Biological Co.LTD. Transfection was performed when the cell fusion reached 50-60%. Successively add: DMEM culture solution (no serum, no double antibody), PEI, plasmid, prompt separation,

mix, and stand in a sterile environment for 20min; Incubate in a 5% CO_2 incubator at 37 ℃ for 4-6h; After 4-6h, discard the transfer solution, add DMEM culture solution (no serum, no double antibody) to the labeled 6-well plate or petri dish, respectively, and incubate in the incubator (37 ℃, 5% CO_2) overnight (generally 24h). On the next day, 6-well plates or petri dishes were taken out, the culture medium was discarded, washed with PBS for 3 times, and culture medium containing different treatments prepared according to the needs of the groups was added to them respectively, and incubated in the incubator (37 ℃, 5% CO_2) for 48h. The cells were divided into the following groups: normal glucose control group (NG), high glucose control group (HG), normal glucose STAT3 no-load and plasmid treatment group, and high glucose STAT3 no-load and plasmid treatment group.

2.3. Materials and Reagents

Rhubarb (Chang Zhongjing Pharmacy, Henan, Lot No.: 220902), aloe-emodin (MCE), mouse anti-STAT3 (Abcam), rabbit anti-phospho-STAT3 (CST), Mouse-anti-IL-1β ELISA (Elabscience), Mouse-anti-IL-18 ELISA (Elabscience), Rabbit-anti-CollagenIII (Sigma), Mouse-anti-E-cadherin (Proteintech), Mouse-anti-Caspase-11 (Santa Cruz), Rabbit-anti-GSDMD (Abcam), STAT3 plasmid (Shanghai Yi Le biological Company), Urinary microalbumin assay kit, Total cholesterol assay kit, Triglyceride determination kit, Creatinine test kit.

2.4. Database Screening of Main Components and Gene Targets of Rhubarb

The active components of Rhubarb extract were screened using TCMSP according to oral bioavailability (OB) ≥ 30% and drug-likeness (DL) ≥ 0.18. Then the Swiss Target Prediction database was used to query and screen out all the gene targets corresponding to the active components of Rhubarb extract, and the target protein was corrected as the gene name by UniProt database after the repeated targets were removed.

2.5. Obtain Dkd Related Gene Targets

On Gene Cards (https://www.genecards.org/) and OMIM (http://www.omim.org) DKD related gene targets are retrieved with the keyword "Diabetic Kidney Disease, DKD" in the database. After collection, repeats are removed, and the intersection targets between rhubarb active ingredients and diseases are obtained through Venn diagram.

2.6. Construction of Protein-Protein Interaction Network

The protein-protein interaction (PPI) between potential targets of rhubarb active ingredients and DKD targets was analyzed through the STRING database, and the protein-protein interaction relationship network was constructed. The relevant parameters are set to: Species is set to Human species, reliability >0.40, and the rest are default values. Delete the discrete target and download the TSV file to import Cytoscape software to map the PPI network. Then the core active ingredients and targets of rhubarb extract for DKD were screened according to the node color depth and Degree.

2.7. Go and Kegg Pathway Enrichment Analysis

The core targets were imported into the DAVID (https://david.ncifcrf.gov/) database for GO and KEGG enrichment analysis. The species "Homo sapiens" was set, and the screening criteria was $P<0.01$. Then enrichment results through microscopic letter platform (http://www.bioinformatics.com.cn) for the main biological processes and metabolic pathways for visualization, analysis the main biological processes involved and signaling pathway and action mechanism of the rhubarb treatment DKD.

2.8. Construct a "Compound - Target - Pathway" Network Diagram

Construct an "active Compound - Target - pathway" network including active compounds, potential targets and pathways of Rhubarb using Cytoscape software. The color depth of the node is designed according to the degree value.

2.9. Molecular Docking

Download the MOL2 format of two-dimensional structure of core components from TCMSP or Pub Chem database, and then download the PDB format of core targets from PDB (https://www.rcsb.org) database. Auto Dock Tools were used to dewater and hydrogenate the downloaded target protein receptors and ligand small molecules, and then exported and saved as pdbqt file. Docking parameters and docking range were set, and molecular docking of active ingredients and key targets was performed with Auto Dock Vina. The lowest binding energy conformation was saved, the corresponding visualization results were displayed using Pymol software, and the binding energy heat map was constructed using Weisenxin platform software.

2.10. Molecular Experimental

Total cholesterol and triglyceride detection: mixed according to the instructions, incubated at 37 ℃/10min at 510nm wavelength, and the absorbance value of each hole was determined by enzymolysis. TNF-α, IL-18, IL-1β, creatinine and mouse urinary microalbumin were measured according to the instructions.

The animal protein samples were quantitatively analyzed

according to the instructions of the BCA assay kit, and then the proteins were denatured, followed by electrophoresis, membrane transfer, sealing, and primary antibody incubation overnight. The next day, the second antibody was incubated, and finally the development and exposure were performed. The grayscale value of each strip was obtained using Quantity one software (β-actin as the internal parameter), and the relative expression of the target protein was calculated. Western Blot exposure strip gray value processing software Image Lab 4.0 and Quantity one software, data statistical analysis software SPSS21.0, statistical chart making software GraphPad Prism7.0. Statistical data were expressed as mean ± standard deviation ($\bar{x} \pm s$), paired T-test was used for comparison between two groups, and one-way analysis of variance was used for comparison between multiple groups. P <0.05 indicated statistically significant differences.

3. Results

3.1. Compounds And Targets of Rhubarb

TCMSP database was used to screen the active ingredients in Rhubarb extract, and the active ingredients were screened according to OB≥30% and DL≥ 0.18 (Table 1). The Swiss Target Prediction database was used to screen all gene targets corresponding to the active components of Rhubarb extract. 13,763 DKD-related Gene targets were obtained from the Gene Cards and OMIM databases, and a total of 343 intersection targets were obtained with the active ingredients of drugs. In view of the fact that a large number of intersection targets was not conducive to the prediction and analysis of subsequent results, the follow-up study was finally conducted with 121 targets greater than the average value (Table 2).

Table 1. *TheMolID, OB and DL of compounds in DKD.*

MOL ID	Molecule Name	OB (%)	DL
MOL002235	EUPATIN	50.8	0.41
MOL002259	Physciondiglucoside	41.65	0.63
MOL002268	rhein	47.07	0.28
MOL002281	Toralactone	46.46	0.24
MOL002288	Emodin-1-O-beta-D-glucopyranoside	44.81	0.8
MOL002293	Sennoside D_qt	61.06	0.61
MOL002297	Daucosterol_qt	35.89	0.7
MOL000358	beta-sitosterol	36.91	0.75
MOL000471	aloe-emodin	83.38	0.24
MOL000096	(-)-catechin	49.68	0.24

Table 2. *Potential targets of Rhubarb and DKD.*

No.	Target	No.	Target	No.	Target	No.	Target	No.	Target
1	AKT1	26	ABL1	51	SLC2A1	76	HDAC2	101	CCR1
2	STAT3	27	CDK2	52	CYP3A4	77	DRD2	102	PLK1
3	EGFR	28	SYK	53	PRKCD	78	NOS2	103	CDC25C
4	NFKB1	29	PDGFRB	54	PTPN6	79	PDPK1	104	PTGS1
5	SRC	30	CDK1	55	LGALS3	80	CYP19A1	105	ALOX5
6	ESR1	31	LYN	56	HMGCR	81	TYMS	106	ACHE
7	HSP90AA1	32	PIK3CB	57	CTSS	82	OPRM1	107	GRM5
8	MMP9	33	AR	58	KEAP1	83	RET	108	F2
9	GSK3B	34	ACE	59	PTK2B	84	PRKCZ	109	CYP1B1

No.	Target	No.	Target	No.	Target	No.	Target	No.	Target
10	MTOR	35	ABCB1	60	FLT3	85	IDO1	110	SLC6A3
11	PTGS2	36	NOS3	61	ZAP70	86	XDH	111	SLC6A4
12	CXCR4	37	PTK2	62	MMP3	87	CDK5	112	G6PD
13	TLR4	38	PRKACA	63	AXL	88	PLAT	113	HTR2A
14	PIK3CA	39	NFE2L2	64	SHH	89	CTSD	114	HPRT1
15	KDR	40	NR3C1	65	ALK	90	CHUK	115	CFTR
16	STAT1	41	ABCG2	66	TOP2A	91	GSTP1	116	CYP17A1
17	IGF1R	42	CDK6	67	MAPT	92	DHFR	117	KDM1A
18	MMP2	43	PIK3CD	68	ARG1	93	PPARD	118	GRK5
19	APP	44	PIK3CG	69	PLG	94	CDC25A	119	GRIN1
20	PIK3R1	45	CTSB	70	CCNE1	95	PSMB9	120	ACACA
21	CASP8	46	MPO	71	ROCK1	96	ELANE	121	CYSLTR2
22	PTPN11	47	SERPINE1	72	PTPN1	97	CYP2C19		
23	EZH2	48	PDGFRA	73	NOX4	98	BACE1		
24	ITGB1	49	CHEK1	74	MAOA	99	CSNK2A1		
25	MET	50	ESR2	75	INSR	100	CXCR1		

3.2. "Active ingredient-target protein" Network

A Venn diagram of DKD and rhubarb targets was made on the Weishengxinxin visual analysis platform. There were 343 intersection targets between DKD and Rhubarb in total (Figure 2A). The "Active ingredient - target protein" network was constructed using string database and Cytoscape software based on a larger than average 121 target sites (Figure 2B-C).

3.3. Go Functional Enrichment Results

The common intersection target genes were imported into the DAVID database, and then mapped through the Weishengxinxin visual analysis platform, and the functional enrichment results of GO and KEGG were obtained. The GO enrichment results showed that the Biological Progress (BP) of rhubarb in treating DKD mainly included: protein phosphorylation, positive regulation of protein kinase B signaling, protein autophosphorylation, transmembrane receptor protein tyrosine kinase signaling pathway, peptidyl-tyrosine phosphorylation, etc. The enrichment of Cell components (CC) involved mainly concentrated on: cytoplasm, membrane raft, plasma membrane, macromolecular complex, cytosol, etc. protein serine/threonine/tyrosine kinase activity, ATP binding, protein kinase activity, protein tyrosine kinase activity, enzyme binding, etc. The results suggest that DKD is involved in multiple gene functions, and rhubarb may play a role in treating DKD by regulating these biological processes (Figure 3A-D).

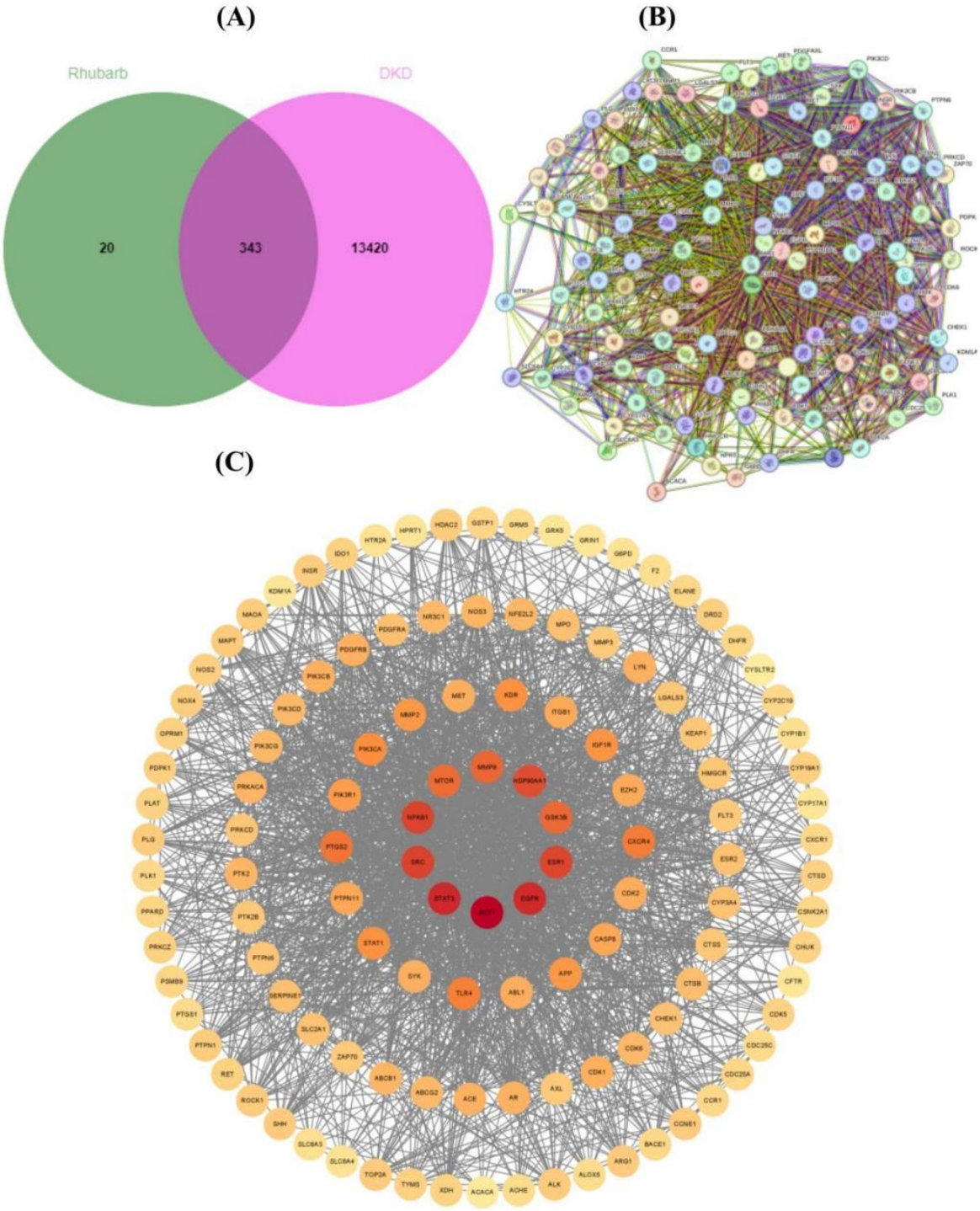

Figure 2. *Active ingredients and targets of Rhubarb. (A) Venn diagram showing intersection targets of rhubarb and DKD. (B) string diagram shows the network results of protein-to-protein interactions. (C) "Active ingredient-target protein" network diagram. The color gradient from the inner ring to the outer ring varies according to the degree value, and the edge indicates the interaction between proteins.*

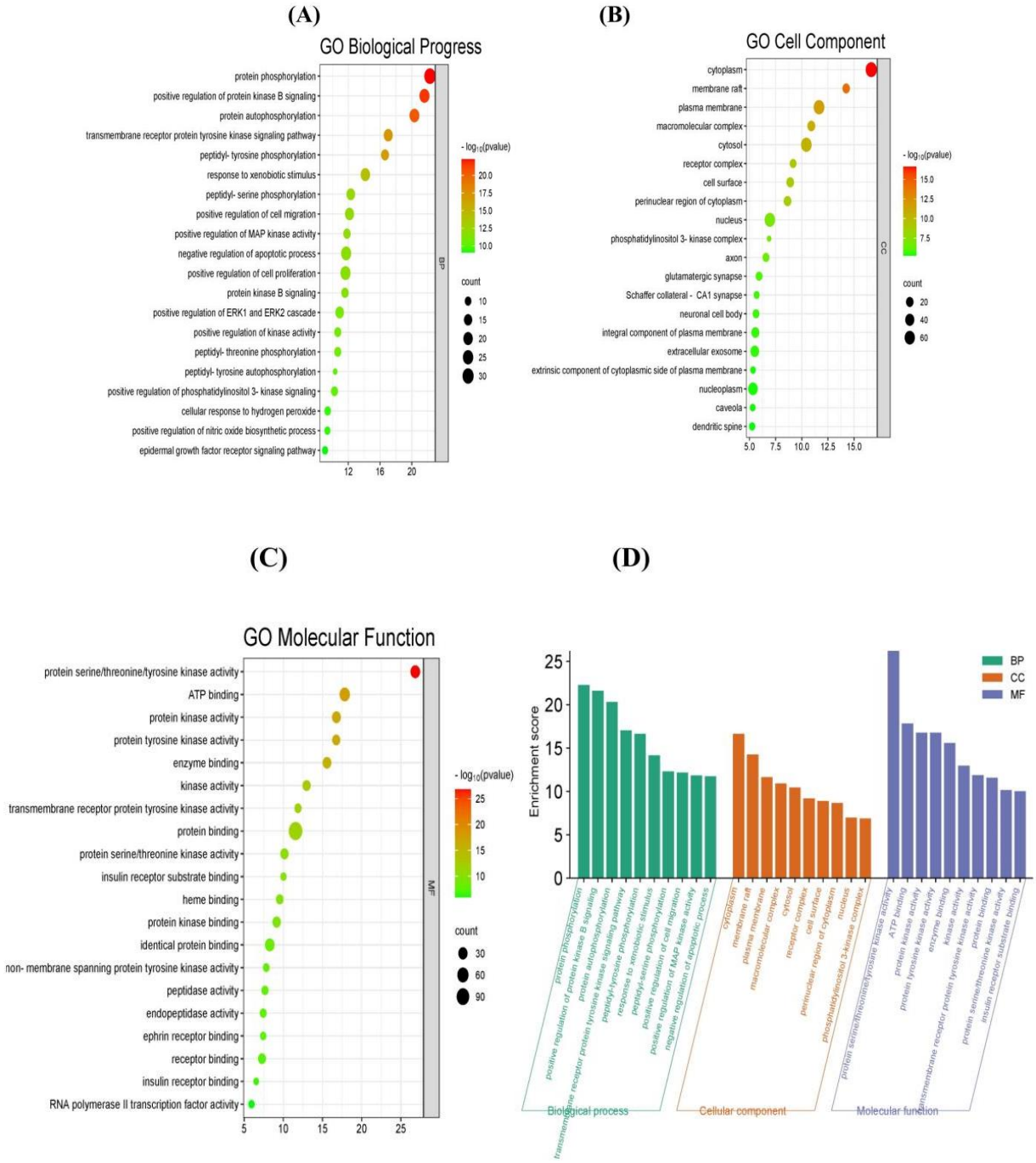

Figure 3. *GO enrichment analysis results. (A) Biological processes (BP). (B) Bubble map of cell composition (CC). (C) Molecular function bubble diagram (MF). The color and size of each bubble is based on the P-value and the number of genes, respectively. (D) GO enrichment analysis. In the first ten enrichment diagrams, green, orange, and purple represent enrichment analysis biological processes, cell components, and molecular functions.*

3.4. KEGG Functional Enrichment Results

Analysis of KEGG enrichment results showed signal Pathways (the top 20 signal pathways were selected): including Pathways in cancer, Prostate cancer, Proteoglycans in cancer, Chemokine signaling pathway, PI3K-Akt signaling pathway, etc. It is suggested that rhubarb may achieve therapeutic effects on DKD through these pathways (Figure 4A-B). Network and type files were prepared respectively for pharmaceutical chemical components, higher-than-average intersection targets, and KEGG enriched results, and were uploaded to Cytoscape software to construct the "component-target-pathway" network diagram (Figure 4C). The visual analysis platform of Weishengxin was used to draw the Sankey diagram, and the results showed that one Target gene corresponded to multiple channels, and one channel corresponded to multiple Target genes (Figure 4D). The re-

sults of KEGG enrichment showed that cancer signaling pathway ranked first, involving a variety of pathways, such as Jak-STAT signaling pathway, PI3K-Akt signaling pathway, Wnt signaling pathway, VEGF signaling pathway, and mTOR signaling pathway, which affect cell proliferation, apoptosis, and gene damage, etc. (Figure 5).

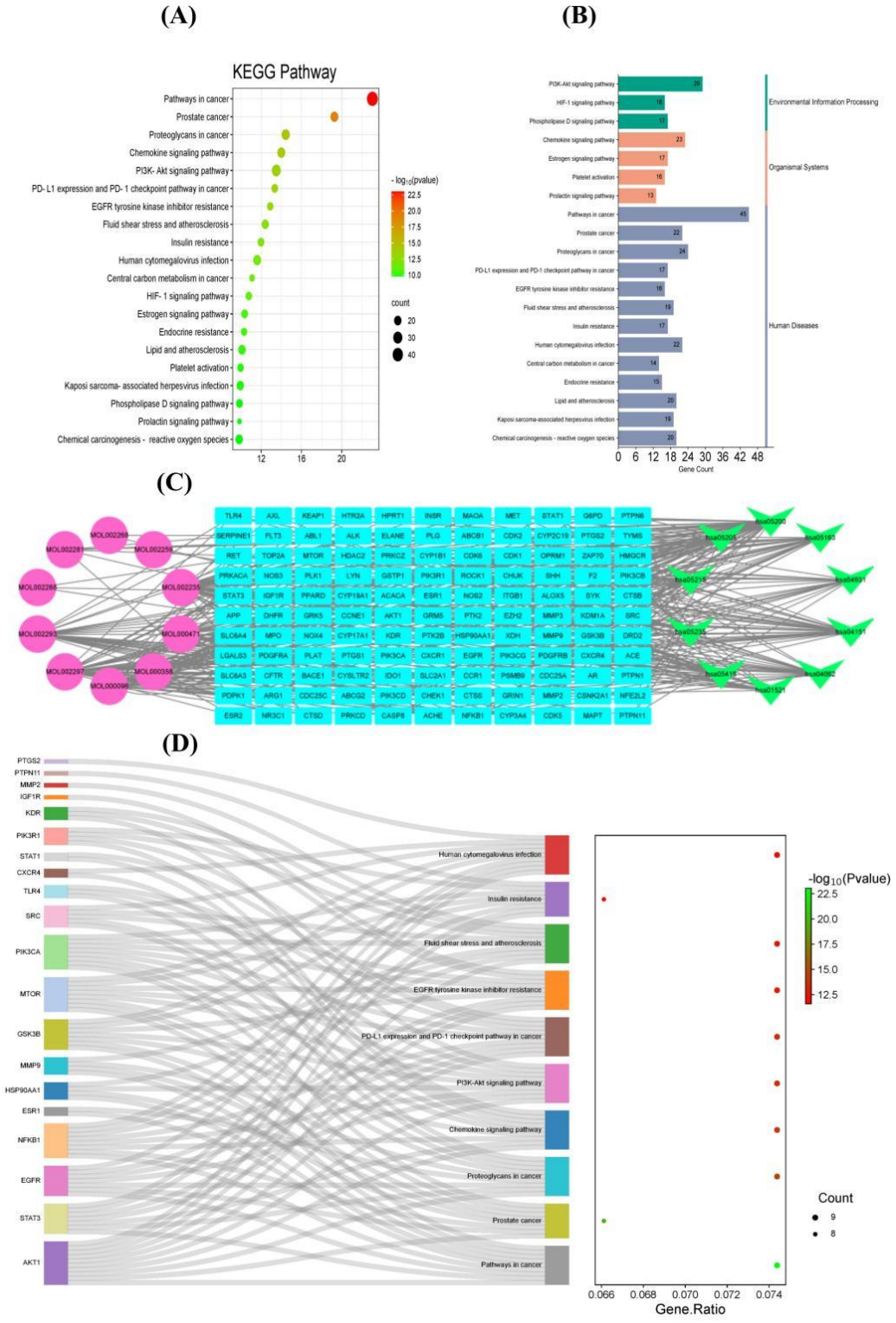

Figure 4. *KEGG enrichment results and "component-target-pathway" network diagram. (A) Bubble maps of the top 20 pathways of KEGG enrichment analysis. (B) Classification of the top 20 pathways. (C) The "ingredient-target-pathway" network diagram involved in the mechanism of rhubarb treatment of DKD: the purple circle, the blue quadrilateral and the green V-shaped represent the active drug ingredients, targets and pathways enriched in Rhubarb, respectively. (D) KEGG's Sankey result graph quantifies the linear flow ratio of incoming/outgoing data for the same node.*

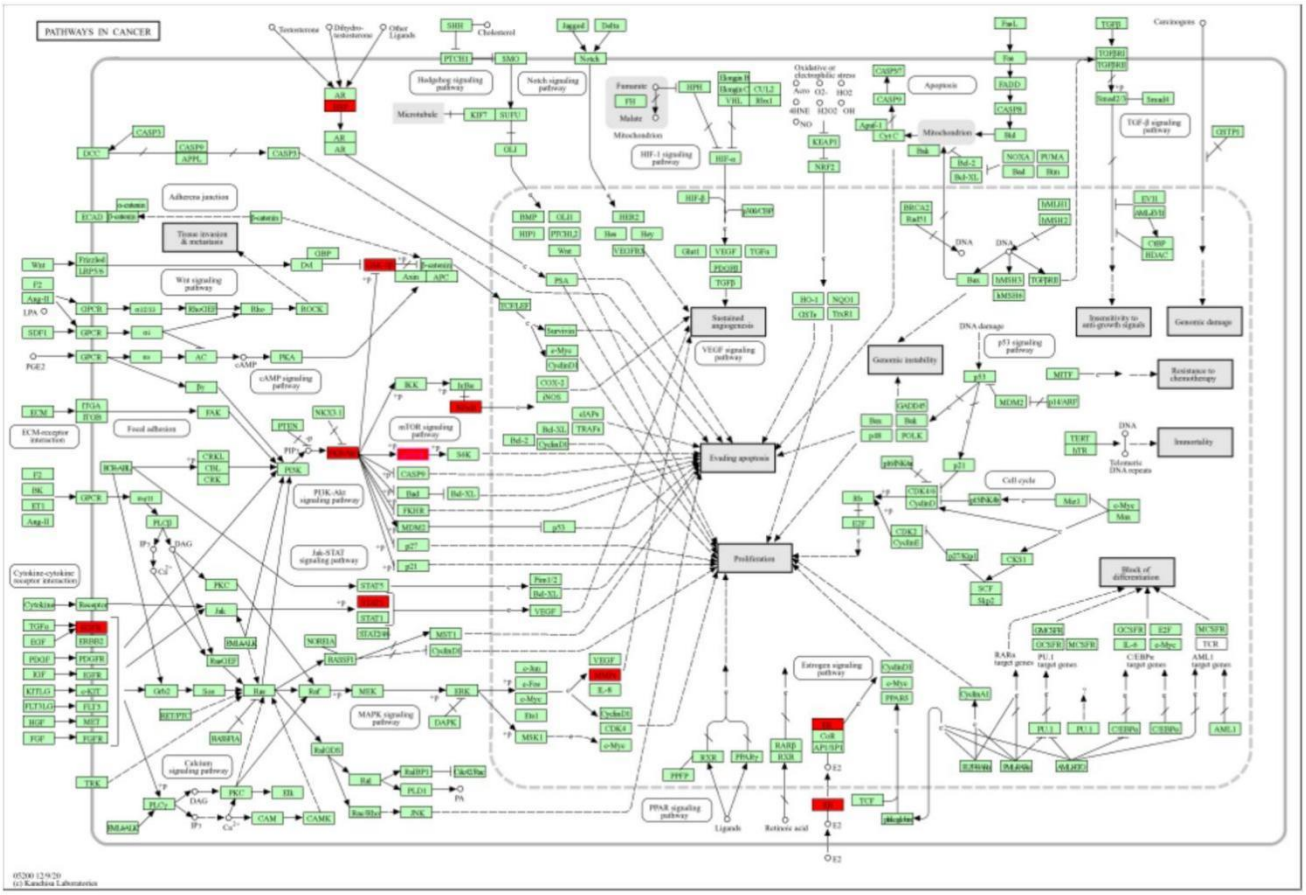

Figure 5. *The distribution of key targets in Pathway in cancer signaling pathway.*

3.5. Molecular Docking

According to the results of PPI analysis, AKT1, STAT3, EGFR, NFκB1, SRC with a higher degree were selected to combine with EUPATIN, Physciondiglucoside, rhein, Toralactone, the main active ingredient of rhubarb. Emodin-1-O-beta-D-glucopyranoside, Sennoside D, Daucosterol, beta-sitosterol, aloe-emodin, (-) -Catechin were sequentially simulated for molecular docking. The results of molecular docking indicate the binding activity between the receptor and the ligand in terms of the binding energy. The smaller the binding energy, the higher the affinity between the receptor and ligand, and the greater the possibility of interaction. Binding energy <−5.0kJ/mol indicates high binding activity, and binding energy <−7.0 kJ/mol indicates strong binding activity. Visualization analysis was performed by Pymol software, and the binding sites were analyzed by PLIP website. According to the selected compounds and target proteins sorted by degree, we focused on aloe-emodin and STAT3. STAT3 and aloe-emodin were combined through hydrophobic interaction, hydrogen bonding and salt bridge, and the binding energy of -7.5 indicated strong binding activity. According to the degree values obtained in PPI, the binding ability of the first 5 core proteins and the active ingredients was predicted, and the heat map was drawn according to the binding energy score (Figure 6A-B).

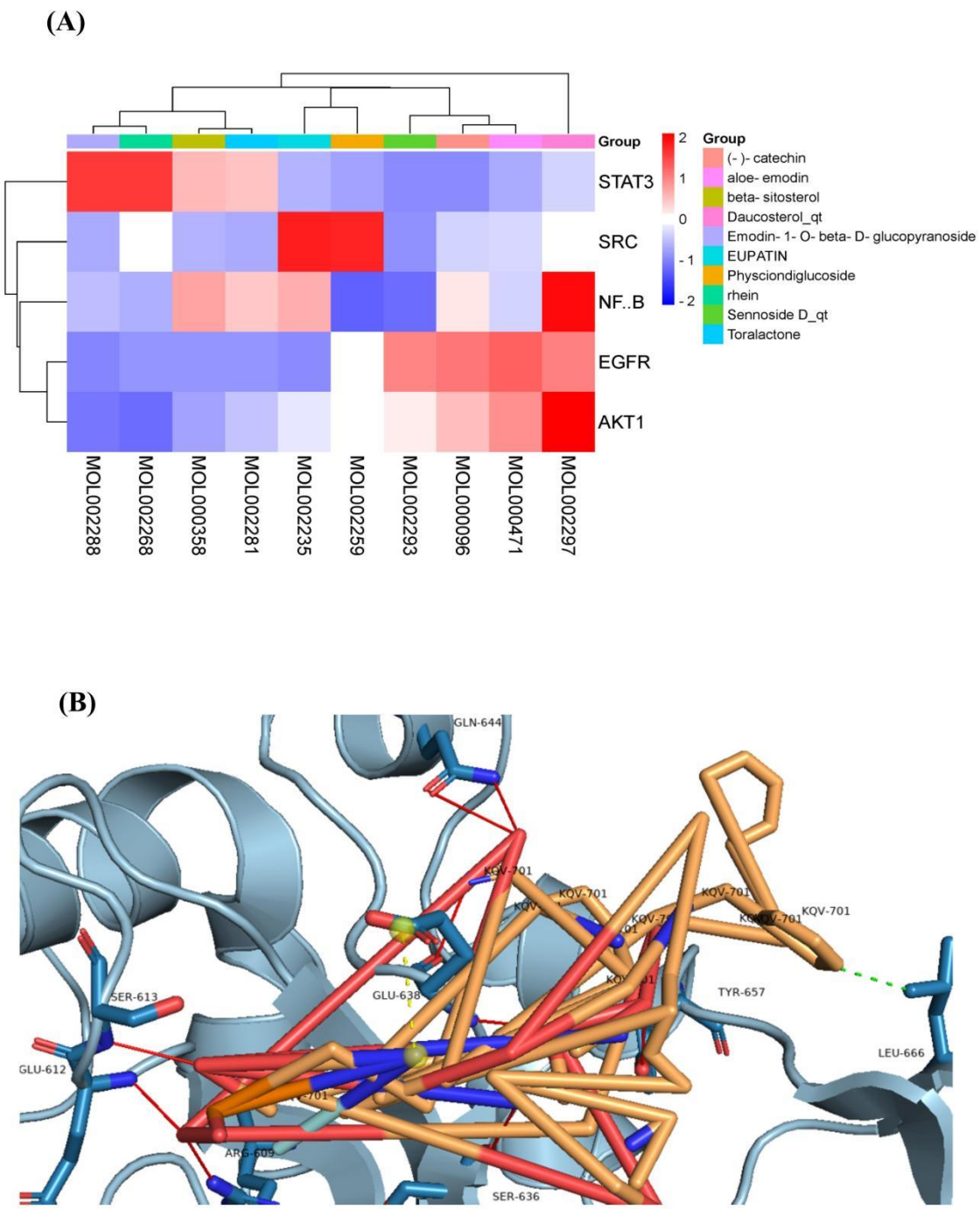

Figure 6. *Molecular docking (A) Heat map of binding energy score of active ingredient and target protein (B) The results of molecular docking between AE and STAT3.*

3.6. Detection of Renal Function Indicators

After gavage of rhubarb (3g•kg-1) and AE (10mg/kg), the overall state of the mice was better than that of the db/db group, with no death and decreased BG, and the MAU, CRE and UACR of the mice were decreased (Figure 7A-D), T-CHO and TG were decreased. Both IL-18 and IL-1β were decreased (Figure 7E-H). The difference was statistically significant ($P<0.05$).

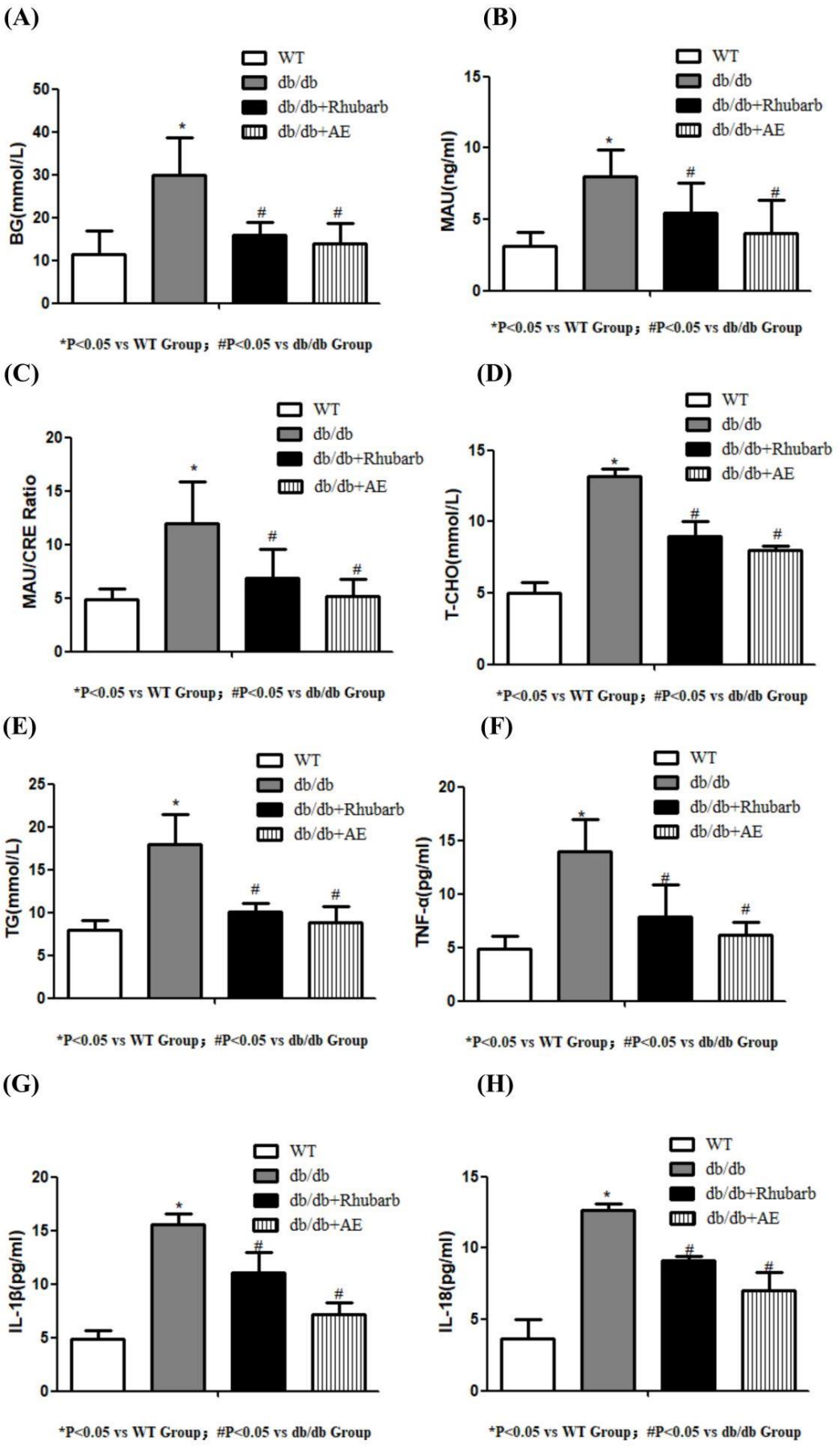

Figure 7. *Biochemical index detection (A) Mouse blood glucose statistics (B) Urine microalbumin test results (C) CRE test results (D) UACR ratio test results. Results of T-CHO (E), TG (F), IL-1β (G) and IL-18 (H) in mouse.*

3.7. Western Blot and Co-immunoprecipitation

Compared with WT group, the expressions of *P*-STAT3, STAT3, Caspase-11 and GSDMD-N in kidney tissues in db/db group have been significantly increased, while the expressions of Collagen-III and α-SMA in db/db group have been significantly increased, and the expression of E-cadherin protein in db/db group has been decreased. After Rhubarb and AE intervention, the expression of E-cadherin protein increased, and the other indexes decreased. The difference was statistically significant ($P<0.05$), while GSDMD-FL did not change (Figure 8A-D). Verification of the interaction between STAT3 and Caspase-11: Co-immunoprecipitation results indicated that there was a protein-protein interaction between STAT3 and Caspase-11 (Figure 8E).

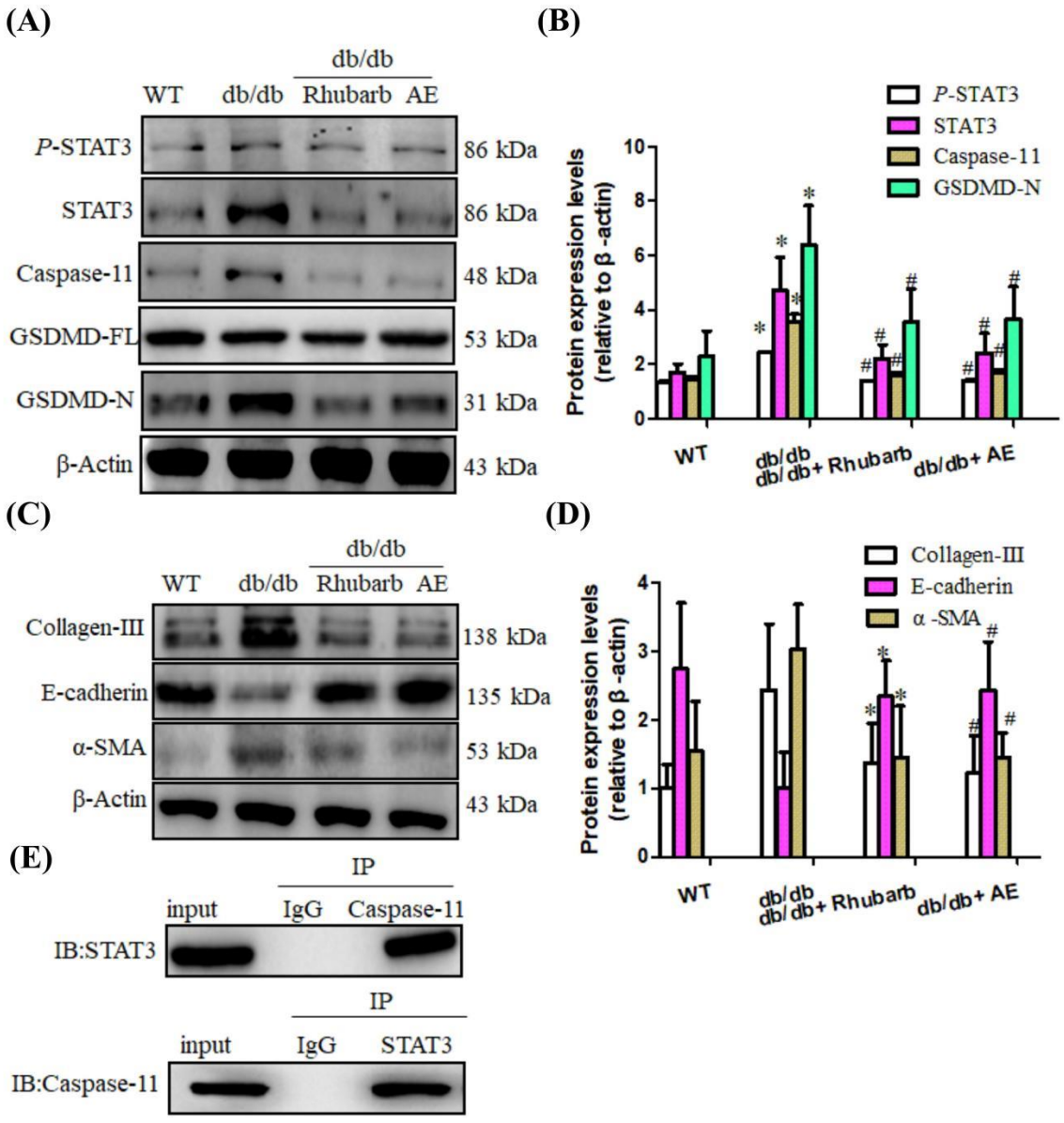

Figure 8. *Western blot test results The expression of pyroptosis (A-B) and fibrosis indexes (C-D) was detected in vivo, and the interaction between STAT3 and Caspase-11 was detected by co-immunoprecipitation (E).*

3.8. Cell Experiment

After knocking down STAT3, the indicators of pyroptosis and fibrosis were detected. The results showed that the protein of each group was collected for Western blot detection. The results showed that the expressions of Caspase-11 and GSDMD-N decreased significantly after STAT3 knockdown, The expression of Collagen-Ⅲ and α-SMA protein has been

down-regulated, while the expression of E-cadherin protein has been recovered, The difference was statistically significant, $P<0.05$ (Figure 9A-D). After STAT3 overexpression, Western blot analysis of the indicators of pyroptosis and fibrosis showed that: The expressions of Caspase-11 and GSDMD-N increased significantly after overexpression of STAT3. The expression of Collagen-Ⅲ and α-SMA protein was up-regulated, The expression of E-cadherin protein was significantly decreased, and the difference was statistically significant, $P<0.05$ (Figure 9E-H).

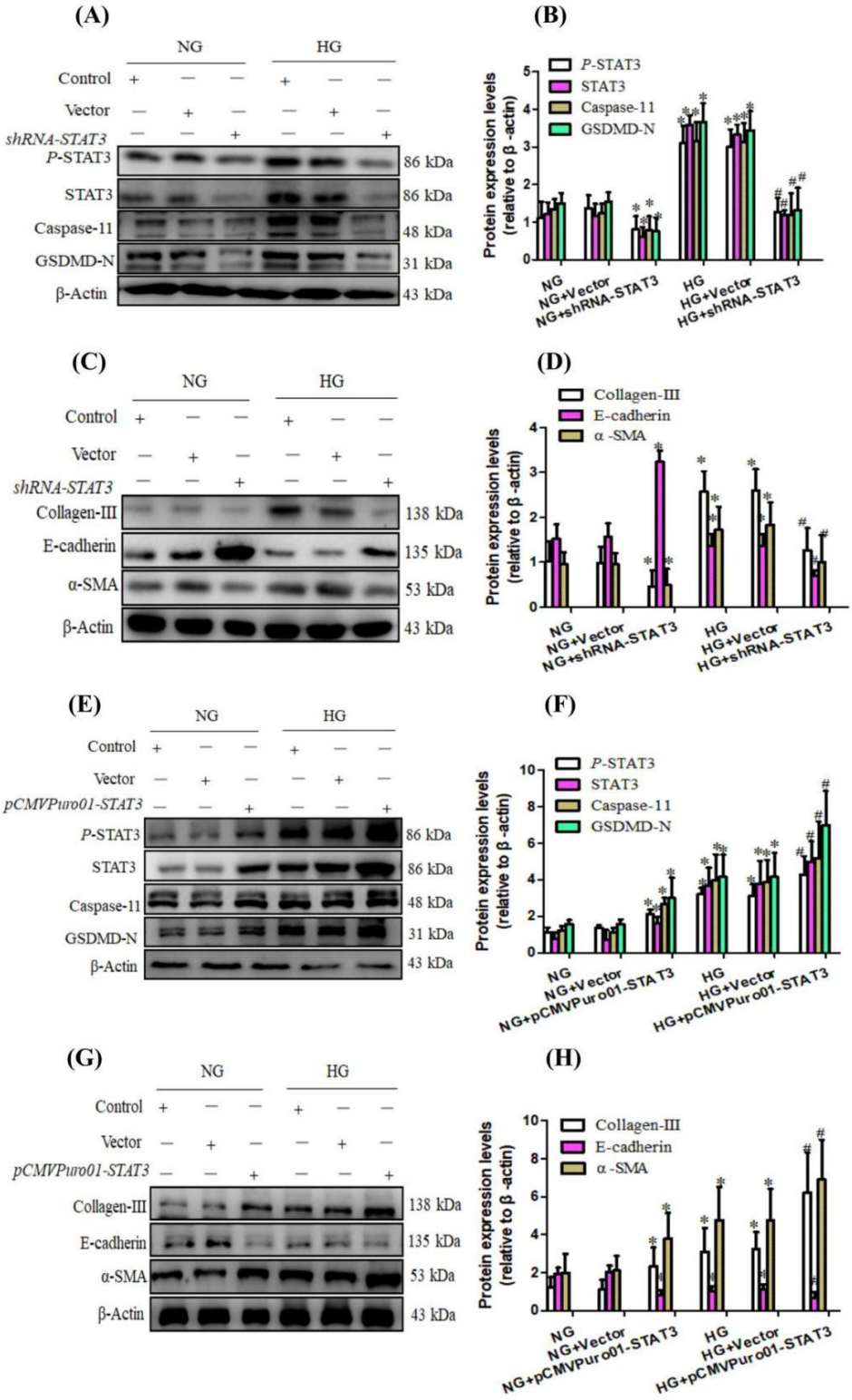

Figure 9. *Effects of STAT3 knockdown on pyroptosis (A-B) and fibrosis indexes (C-D). Effects of STAT3 overexpression on pyroptosis (E-F) and fibrosis indexes (G-H).*

4. Discussion

DKD is a common complication of diabetes, its pathogenesis is very complex, modern medical treatment can not effectively control the progress of the disease. TCM (TCM), which has been passed down for thousands of years, is safe and effective in treating diabetes through multi-target and multi-factor treatment. Diabetes belongs to the category of "the dispersion-thirst disease" in TCM. The lesion of viscera involves many parts such as lung, stomach and kidney. The physiological functions of kidney include human growth, development, excretion and reproduction. The effect of rhubarb is mainly for purging and attacking accumulation, clearing heat and purging fire, detoxifying, promoting blood circulation and removing blood stasis. Rhubarb has a good purging effect and has a good effect in the treatment of DKD [15, 16].

Network pharmacology is to search and screen the active ingredients of drugs on the pharmacology database and analysis platform of TCM system. The target genes corresponding to effective chemical components were intersected with disease-related genes. The corresponding signaling pathway was obtained by intersecting target proteins, and the network model of "active ingredient-target-signaling pathway" was established to show the result of the effective active ingredient against the disease through the possible pathway. Thus, the interaction between drug ingredients and disease can be clarified. Compared with the traditional pharmacological experiments, the multi-pathway regulation of the signal pathway can improve the therapeutic effect of drugs and reduce the side effects of drugs.

In this study, 10 active ingredients of Rhubarb were collected by network pharmacology method. The PPI network was constructed by STRING database and Cytoscape software, and AKT1, STAT3, EGFR, NFKB1 and SRC were selected as core targets. According to molecular docking verification, most of the above core targets and the main active components of rhubarb have good binding ability and stable conformation through molecular docking simulation in sequence, and it is speculated that the above core targets are the key targets for rhubarb to interfere with DKD.

Aloe emodin (Rhabarberone), a major active ingredient in Rhubarb, has anti-tumor activity and induces apoptosis by playing an anti-proliferative role. Studies have found that AE can affect the development of some diseases by regulating pyroptosis. But the specific role of AE in DKD is unclear [17-19]. Studies have shown that STAT3 can affect the progression of disease by regulating the pyroptosis of cells [20-22]. Other studies have shown that STAT3 can interfere with the process of DKD in kidney diseases regardless of whether it is an upstream regulatory factor or a downstream target protein [23-26].

In summary, in the process of DKD, is there a mechanism by which AE affects pyroptosis in DKD by regulating STAT3? The results of molecular docking showed that STAT3 and AE were combined through hydrophobic interaction, hydrogen bonding and salt bridge, and the binding activity was very strong. It is suggested that AE may combine with STAT3 to achieve the purpose of DKD treatment. Interestingly, after the AE intervention of rhubarb and one of its active ingredients. we found that the changes in the indicators were basically the same. Therefore, the potential drug effects of AE on DKD need to be further explored.

5. Conclusions

In conclusion, the intersection targets of rhubarb active compounds and DKD were identified by network pharmacology in this study. After analyzing the results of PPI database and molecular docking technology, this study focused on STAT3 protein and active component AE. Subsequently, molecular biology experiments were used to further verify the relationship between the two, and the final results showed that rhubarb and AE affected the mechanism of pyroptosis in DKD by regulating the STAT3/Caspase-11 axis. This provides a basic experimental basis for exploring the potential effect of rhubarb in the treatment of DKD and the clinical treatment of DKD.

Abbreviations

DKD	Diabetic Kidney Disease
TCM	Traditional Chinese Medicine
AE	Aloe emodin

Author Contributions

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Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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Biography

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Research Field

Yanwen Mao: Pathogenesis of chronic kidney disease and traditional Chinese medicine prevention and treatment; Neurodegenerative diseases and traditional Chinese medicine prevention and treatment

Minghao Zhang: Pathogenesis of chronic kidney disease and traditional Chinese medicine prevention and treatment; Neurodegenerative diseases and traditional Chinese medicine prevention and treatment

Zijuan Zhang: Neurodegenerative diseases and traditional Chinese medicine prevention and treatment

Xiaowei Zhang: Pathogenesis of chronic kidney disease and traditional Chinese medicine prevention and treatment

Wenhui Rong: Cardiovascular system disease and traditional Chinese medicine prevention and treatment

Juan Zhang: Pathogenesis and prevention of liver fibrosis with traditional Chinese medicine

Mengmeng Yang: Chronic lung diseases and traditional Chinese medicine prevention and treatment

Jiangyan Xu: Pathogenesis of chronic kidney disease and traditional Chinese medicine prevention and treatment; Neurodegenerative diseases and traditional Chinese medicine prevention and treatment; Cardiovascular system disease and traditional Chinese medicine prevention and treatment