

Predicting the mechanism of action of Silymarin —Pueraria lobata—Prednisolone combination in the treatment of alcoholic liver disease based on network pharmacology and molecular docking.

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Abstract. Objective: To predict the key targets, active ingredients, and pathways of action of Silymarin-Pueraria lobata- Prednisolone combination for the treatment of Alcoholic Liver Disease (ALD) based on the web-based pharmacology and molecular docking approach, and to determine the biological processes and molecular functions of its action. Methods: Several databases including TCMSP, Pharm Mapper, PubChem, TTD, Drugbank, OMIM and UniProt were used to identify the targets of Silymarin, Pueraria lobata and Prednisolone for the treatment of ALD. The main targets of the combination of Chinese and Western medicines were identified by Venn diagram analysis. Protein interaction networks were constructed and information on biological processes and molecular functions was obtained with the help of the STRING web site. Target genes were visualised using the Cytoscape software. Potential targets were analysed using the Metascape website. Molecular docking was performed with the key target genes screened. This was done to confirm the drug compounds identified in the enrichment results. Results: A total of 95 targets were identified in the combination therapy of Silymarin-Pueraria lobata-Prednisolone for the treatment of ALD. The key targets include ALB, IL6, and TNF. When used for treating ALD, the therapy primarily acts on the following functions: Chemical carcinogenesis - receptor activation, Neuroactive ligand-receptor interaction, Pathways in cancer, and Calcium signaling pathway, among others. In the treatment of ALD, 161 pathways are primarily targeted, including those involved in blood circulation, cellular response to organic cyclic compounds, cationic acid, and various other pathways. These pathways include response to organic cyclic compounds, circulatory system processes, nuclear receptor activity, ligand-activated transcription factor activity, neurotransmitter activity, and neurotoxicity, as well as neurotransmitter receptor activity, and 157 other molecular functions for the combination therapy of ALD. Research using molecular docking techniques indicates that the combination therapy of Silymarin, Pueraria lobata and Prednisolone is effective in the treatment of ALD due to the high affinity observed between the core target proteins and the active ingredients of the drug. Conclusion: The primary objective of combining Silymarin, Pueraria lobata, and Prednisolone therapy for ALD was screened and confirmed via network pharmacology and molecular docking. The analysis also predicted the possible mechanism of action for the combination of Chinese and Western medicines in ALD treatment, laying a solid foundation for further development of Silymarin-Pueraria lobata-Prednisolone therapy. Providing a theoretical framework for the subsequent experimentation and clinical validation of Silymarin-Pueraria lobata-Prednisolone combination therapy for ALD. Concurrently, it serves as a reference for enhancing network pharmacology and molecular docking methodologies when predicting the mechanism of actions of combined Chinese and Western therapies for various diseases.

Keywords: Silymarin-Pueraria lobata-Prednisolone combination therapy; alcoholic liver disease; network pharmacology; molecular docking.

1. Introduction

Alcoholic liver disease (ALD) is a condition caused by prolonged heavy alcohol consumption, resulting initially in the development of fatty liver, progressing to alcoholic hepatitis, liver fibrosis, cirrhosis, and potentially liver cancer. Alcohol consumption is the primary causative factor. However, many other factors contribute to the pathogenesis of the disease. After ethanol enters the hepatocyte,

it undergoes a process of conversion into ethanol dehydrogenase through hepatic ethanol dehydrogenase, which is an enzyme responsible for the catabolism of hydrogen peroxide as well as hepatic microsome ethanol oxidation. This results in the formation of acetaldehyde which significantly exhibits hepatocyte toxicity causing metabolic blockage, ultimately leading to liver cell degeneration and necrosis. Alcoholic liver disease is amongst the most prevalent liver diseases. Alcoholic liver disease is a prevalent liver condition, and its incidence in hospitalized patients has been increasing in recent years, posing severe risks to public health. Complete abstinence from alcohol and the supplementary medication, baclofen, constitute the fundamental treatment approach for alcoholic liver disease. However, many patients face challenges when trying to stop drinking, experience repeated drinking episodes, and have poor recovery outcomes. In addition, the adjunct medication baclofen poses serious toxicity risks to various bodily systems, including the central nervous, digestive, and cardiovascular systems.

Combination therapy for alcoholic liver disease (ALD) has shown great potential with the widespread use of traditional Chinese medicine (TCM) worldwide, the identification of key targets and their mechanisms of action, and the increasing integration of Chinese and Western medicine [1]. According to the Compendium of Chinese Herbal Medicines and the National Compendium of Chinese Herbal Medicine, Silymarin has demonstrated efficacy in reducing liver injury, preventing atherosclerosis and cerebral ischemia, inhibiting platelet aggregation, and fighting against tumours. In the Compendium of Chinese Herbal Medicine, silymarine is reported to be effective against liver damage, atherosclerosis, cerebral haemorrhage, platelet aggregation, and cancer, and is used for rashes. The text already adheres to the principles or lacks context, so the improved version is: Shengji quenches thirst the functions of Pueraria lobata in relieving muscle fever, transmitting rashes, generating body fluid and quenching thirst, elevating yang and stopping diarrhea, activating meridians and collaterals, and detoxifying alcohol are explained in detail. Recent research indicates that the Silymarin-Pueraria lobata complex preparation has a beneficial protective effect against alcohol-induced fatty liver and hepatitis and has potential as a functional drug for the management of alcoholic liver fibrosis [2]. The glucocorticoid Prednisolone exhibits heightened activity compared to cortisol, as it is active without hepatic conversion, and is rapidly and completely absorbed orally. Prednisolone's toxic effects are relatively low. The combination of Silymarin-Pueraria lobata-Prednisolone is anticipated to play a distinctive function in the treatment of alcohol-induced liver disease.

Currently, experimental research on drugs and disease solely concentrates on 'single target-single pathway-single drug', without accommodating multi-targets, multi-pathways, and multi-components, as well as the intricate network relationship between target-component-pathway. Traditional experimental methods tend to be expensive, take an extended cycle time, and have a limited controllability rate, impeding the progress of drug research on alcoholic liver disease. Network pharmacology is an emerging method with advantageous attributes such as affordability, efficiency, and reproducibility. Bioinformatics technology is employed to explain the pathogenesis of alcoholic liver disease, based on the "drug-disease-target-component-pathway" interactions network. Accurate prediction of the disease drug targets enables the overall dynamic drug mechanism prediction, thus providing a novel perspective to explore the therapeutic mechanism of Chinese and Western medicines for alcoholic liver disease. Molecular docking technology represents a novel approach to exploring the mechanism of drug action when employing a combination of Chinese and Western medication to manage alcoholic liver disease. Molecular docking technology proves to be significant in demonstrating the possibility of merging Chinese and Western medications in addressing alcoholic liver disease. This method works by ensuring geometrical and energetic conformity between the disease target and active drug ingredient, along with calculating the affinity between the protein and small molecule. This process ultimately helps in determining the effectiveness of the drug's active ingredient.

Therefore, in this study, the network pharmacology method was selected to identify the pertinent targets and active ingredients of the Silymarin-Pueraria lobata combination for treating alcoholic liver

disease. Bioinformatics analysis was utilised to predict significant biological processes, cellular functions and vital pathways. Additionally, the molecular docking method was employed to authenticate the critical targets and pathways of the amalgamation of traditional Chinese and Western medications in the therapy of alcoholic liver disease. Ultimately, this study proposes innovative ideas for the development of therapeutic drugs and clinical treatment to combat alcoholic liver disease.

2. Material Methods

2.1. Mining of target genes related to Silymarin, Pueraria lobata, and Prednisolone.

The chemical composition of Silymarin and Pueraria lobata was investigated using the Traditional Chinese Medicine Systematic Pharmacology Database and Analysis Platform (TCMSP). For inclusion in the study, only drugs with active ingredients that meet both oral bioavailability (OB) $\geq 30\%$ and drug-like properties (DL) ≥ 0.18 in ADME parameters were selected. The structural formula for Prednisolone was retrieved from the PubChem database and submitted to the Pharm-Mapper database to identify the drug's target of action. The resulting protein targets were then cross-checked with the UniProt protein database to obtain corresponding Uniprot IDs, which were normalised to identify Silymarin-Pueraria lobata co-targets and Prednisolone target genes.

2.2. Screening of targets related to alcoholic liver disease.

Potential targets for alcoholic liver disease (ALD) were screened in the TTD database, supplemented by the OMIM and DRUGBANK databases, with duplicates deleted. The combination of databases was the source of all targets for ALD.

2.3. Construction of protein-protein interaction (PPI) network and 'Silymarin-Pueraria lobata-Prednisolone-ALD' target network

The targets of Silymarin-Pueraria lobata, Prednisolone, and ALD were utilized for creating a Wayne diagram. Afterwards, the shared targets were uploaded into the STRING11.0 database and set as 'Homo sapiens'. A minimum interactions threshold of 'highest confidence' > 0.4 was established. PPI network model and potential modules for cellular function derived by removing unrelated protein targets. The network topology of the PPI network was analyzed using the Cyto NCA plug-in in Cytoscape 3.8.0. We utilized the 'Betweenness value' and 'Degree value' as the common reference number and established the 'Silymarin-Pueraria' network model by designating 'Silymarin-Pueraria' as the common reference number. PPI network topology was analysed using 'betweenness' and 'degree' as a common reference figure, and then 'Silymarin-Pueraria lobata-Prednisolone-ALD' target network was constructed based on this analysis.

2.4. Bioinformatic analysis

The Silymarin-Pueraria lobata, Prednisolone and Alcoholic Liver Disease intersection targets were imported into the Metascape database to obtain the relevant biological information, and the top 10-20 of the top ranked targets were selected for Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, after ranking according to the P value. For Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, the top 10~20 was selected. GO and KEGG enrichment analyses provided biological process (BP), cellular component (CC), molecular function (MF) and pathway information, which were then visualized and processed to obtain enrichment analysis bar charts, bubble plots and Sankey bubble analysis.

2.5. Molecular docking validation

The 3D structures of quercetin, arachidonic acid, stigmasterol, formononetin, beta-sitosterol, as well as the protein crystal structures of the ALD key targets ALB, IL6 and TNF, were obtained by

sorting and screening based on degree value from the respective databases of PubChem (pubchem.ncbi.nlm.nih.gov) and PDB (www.rcsb.org). The 3D structure obtained above, along with the protein crystal structures of significant ALD targets ALB, IL6, and TNF, were retrieved from the Degree database (<http://cadd.labshare.cn>). With the assistance of CB-Dock2 platform (<http://cadd.labshare.cn>), we carried out an analysis of the one-to-one correlation between the aforementioned components and targets. A molecular docking analysis was subsequently performed.

3. Results

3.1. Acquisition of Silymarin-Pueraria lobata, Prednisolone, ALD Targets and Important Active Ingredients

3.1.1 Identification of the Silymarin-Pueraria lobata target and its active ingredient and the Prednisolone target

Following preliminary screening and de-emphasis by ADME, 15 active ingredients of Silymarin-Pueraria lobata were acquired, including quercetin, arachidonic acid, stigmasterol, formononetin, beta-sitosterol, among others, as indicated in Table 1. By amalgamating and de-emphasising the objectives associated with the active components, 218 prospective aims of Silymarin-Pueraria lobata were identified. As illustrated in Table 2, the PharmMapper database recognised 31 targets for Prednisolone.

Table 1 Silymarin-Pueraria lobata active ingredients

MOL ID	Molecule name	OB (%)	DL
MOL001439	arachidonic acid	45.57	0.2
MOL001736	(-)-taxifolin	60.51	0.27
MOL000449	Stigmasterol	43.83	0.76
MOL007180	vitamin-e	32.29	0.7
MOL007449	24-methylidenelophenol	44.19	0.75
MOL007451	silydianin	59.65	0.76
MOL007454	silymonin	81.81	0.8
MOL007455	silandrin	64.14	0.94
MOL007457	isosilychristin	30.32	0.4
MOL000953	CLR	37.87	0.68
MOL000098	quercetin	46.43	0.28
MOL000392	formononetin	69.67	0.21
MOL000358	beta-sitosterol	36.91	0.75
MOL002959	3'-Methoxydaidzein	48.57	0.24
MOL003629	Daidzein-4,7-diglucoside	47.27	0.67

Table 2 Silymarin-Pueraria lobata, Prednisolone targets

Medicinal herb target	Prednisolone target
PTGS1	AKR1C2
PTGS2	MAPKAPK2
RXRA	KIF11
TRPV1	BMP2
RXRG	CA2
SLC6A2	MAPK10
RELA	AR
CCND1	PPIA
MAPK1	CYP19A1

EGF	ALB
CDK2	CASP7
CASP3	TTR
PPARG	HSD17B1
G6PD	HSD17B11
TNFRSF1A	MAPK8
PRKCB	MAPK14
PECAM1	CCNA2
NOS3	HCK
ALOX5	CASP3
PLA2G4A	MAOB
PTEN	CA1
SELP	ESR1
PTGES	ESR2
GLB1	ESRRG
ALDH2	PIM1
ABCA6	MMP3
ALDH3A1	TREM1
UCP2	NR1H2
C1R	BACE1
CETP	GSTP1
ABCG1	PGR
ABCC12	
KCNK10	
TNFRSF1B	
PTGES2	
KCNK2	
COL1A2	
HSP90AB1	
PIK3CG	
PGR	
NR3C2	
NCOA2	
ADH1C	
IGHG1	
NCOA1	
ADRA2A	
SLC6A3	
ADRB2	
AKR1B1	
PLAU	
LTA4H	
MAOB	
MAOA	
PRKACA	
CTRB1	
CHRM3	

CHRM1	
ADRB1	
SCN5A	
HTR2A	
ADRA1A	
GABRA3	
CHRM2	
ADRA1B	
GABRA1	
CHRNA7	
F10	
TOP2A	
F7	
ACHE	
PRSS1	
CALML5	
FGFR4	
AR	
DPP4	
F2R	
KCNH2	
MMP3	
EGFR	
AKT1	
VEGFC	
BCL2	
BCL2L1	
FOS	
CDKN1A	
EIF6	
BAX	
CASP9	
MMP2	
MMP9	
IL10	
RB1	
TNF	
JUN	
IL6	
CDKN2A	
AHSA1	
TP53	
ELK1	
NFKBIA	
POR	
ODC1	
XDH	

CASP8	
TOP1	
RAF1	
SOD1	
PRKCA	
MMP1	
HIF1A	
STAT1	
RUNX1T1	
CDK1	
HSPA9	
ERBB2	
ACACA	
HMOX1	
CYP3A4	
CYP1A2	
CAV1	
MYC	
F3	
GJA1	
CYP1A1	
ICAM1	
IL1B	
CCL2	
SELE	
VCAM1	
PTGER3	
CXCL8	
BIRC5	
DUOX2	
HSPB1	
TGFB1	
SULT1E1	
MGAM	
IL2	
NR1I2	
CYP1B1	
CCNB1	
PLAT	
THBD	
SERPINE1	
COL19A1	
IFNG	
IL1A	
MPO	
NCF1	
ABCG4	

HAS2	
GSTP1	
NFE2L2	
NQO1	
PARP1	
AHR	
PSMD3	
SLC2A4	
COL3A1	
CXCL11	
CXCL2	
DCAF5	
NR1I3	
CHEK2	
INSR	
CLDN4	
PPARA	
PPARD	
HSF1	
CRP	
CXCL10	
CHUK	
SPP1	
RUNX2	
RASSF1	
E2F1	
E2F2	
ACP3	
CTSD	
IGFBP3	
IGF2	
CD40LG	
IRF1	
ERBB3	
PON1	
DIO1	
PCOLCE	
NPEPPS	
HK2	
NKX3-1	
RASA1	
GSTM1	
GSTM2	
NOS2	
ESR1	
PDE3A	
SLC6A4	

ESR2	
MAPK14	
GSK3B	
CHEK1	
PIM1	
CCNA2	
PKIA	
MBLAC1	
IL4	
SIRT1	
ATP5F1B	
MT-ND6	
HSD3B2	
HSD3B1	
DRD1	
GABRA2	
CHRM4	
GABRA5	
CHRNA2	
OPRM1	
MAP2	

3.1.2 Access to ALD-related targets

The TTD database produced 580 targets for ALD, as displayed in Table 2. Upon merging and deemphasising disease targets from the OMIM and DRUGBANK databases, Table 3 exhibits 1,111 ALD targets.

Table 3 ALD-related targets

DRUGBANK target	OMIM target	TTD target
NT5E	AD7CNTP	ALOX5
ADORA1	IBD7	ABCA1
ADORA2A	PLEKHG5	CXCR1
CYP1A2	DJ1	PRKCE
PLCB2	KIF1B	ADRA2C
SERPINA6	MTHFR	PDE4D
CYP3A4	MFN2	ARSB
NR3C1	GBD2	TNFSF13B
SLCO1A2	PINK1	GHR
SELPLG	ECE1	DRD3
ALB	ALPL	ABCC9
SLC22A6	RHD	IFNB1
UGT1A1	RHCE	IL2RA
SLCO1B1	YARS1	IL4R
SLCO1B3	GBD3	PTGS1
SLCO2B1	RNF220	PTGS2
	PARK10	ADA
	PAOD1	LTA4H
	FOXD3	ATP1A1
	DNAJC6	ATP1A2

	IL23R	ATP1A3
	TNNI3K	ATP1B1
	ABCA4	ATP1B2
	AGL	ATP1B3
	DBT	CHRNA1
	ATP1A1	ANO1
	AD13	CCKBR
	NBLST6	PARP1
	NOTCH2NLC	IFNAR2
	ECM1	CYP2A6
	ZNF687	CA6
	MUC1	IL23A
	GBA	CCR4
	PKLR	HPD
	LMNA	NTRK1
	CADM3	ACKR1
	COPA	FLT4
	MPZ	PDE5A
	FMO1	HTR1B
	FMO4	CD79B
	AD14	SLC23A2
	NCF2	JAK2
	CELIAC7	MC2R
	IBD23	GABRG2
	PARK16	ACACB
	INAVA	KCNJ10
	REN	APOB
	IL10	ALPL
	RMD1	TOP1
	TLR5	AGTR2
	PSEN2	CCR5
	ADAM17	PTPRS
	NBAS	CD4
	APOB	CD38
	HADHA	SELP
	MPV17	HTR1D
	ABCG8	AR
	PARK3	ABCC1
	HTRA2	ADRA2A
	FABP1	VKORC1
	CELIAC8	GNRHR
	COPD	LCK
	ZAP70	PRKCA
	LCO	COP1
	IL37	TNNC1
	CHDS2	FNTA
	HOXD10	FNTB

	PDE11A	CA1
	BMP2R	IL12A
	CTLA4	ALDH5A1
	FARSB	CYP19A1
	IRS1	SLC29A1
	SP110	SSTR3
	GIGYF2	RXRA
	ATG16L1	S1PR1
	SAG	ITGB7
	AGXT	TPSAB1
	PDCD1	ERBB2
	IBD9	F8
	MYMY1	FGF23
	CAV3	UGCG
	CX3CR1	CTLA4
	CELIAC9	NRP1
	HSCR6	MMUT
	IBD12	HTR5A
	GBE1	MPL
	CHDS5	FASN
	CBLB	SSTR1
	POGLUT1	DGAT1
	SEC61A1	DIO1
	RAB7	CA12
	AD15	LEPR
	PARK21	DHFR
	ATP2C1	HNMT
	DZIP1L	KCNA5
	GYG1	PLAU
	CELIAC10	FYN
	MME	HSP90AA1
	GNB4	GNRH1
	EIF4G1	SLC46A1
	DNAJB11	CYP2C9
	CELIAC11	FGA
	HTT	FGB
	HDL3	HPGDS
	PROM1	CSF3
	UCHL1	GUCY2D
	PHOX2B	TNF
	AIS4	KCNH2
	HSD17B13	CSF1R
	PKD2	CA2
	PPM1K	ALAD
	SNCA	CHRM4
	ADH1C	VEGFA
	CELIAC6	IDH1

	HSCR9	IDH2
	TRIM2	IDH3A
	GDNF	IDH3B
	IBD18	IDH3G
	HEXB	TNFSF11
	PDE8B	GLA
	APC	CEACAM3
	AITD2	SLC6A2
	CELIAC2	IL17A
	IBD5	DRD1
	PDB4	FCGR2A
	LEAP2	GRIN2A
	SAR1B	CXCL8
	HARS1	CELA1
	LARS1	PGR
	SH3TC2	CYP11A1
	IRGM	JAK3
	SQSTM1	GALNS
	IBD3	VWF
	VAMAS6	TTR
	DHX16	EDNRA
	HLA-B	AHR
	HLA-DQA1	BDKRB2
	HLA-DQB1	SERPING1
	HLA-DPB1	LIPA
	ITPR3	PROC
	AD17	DRD4
	FCYT	BTK
	AITD1	CPS1
	SLC17A5	ABCB1
	COX7A2	EZH2
	ELOVL4	PPP3CA
	BCKDHB	PPIB
	SEC63	ICAM1
	FIG4	GABRA5
	ARG1	AKR1B1
	ENPP1	SRD5A1
	CELIAC12	SRD5A2
	LPA	SRD5A3
	PRKN	GABRD
	TBP	PRSS1
	IL6	SLC6A4
	HNRPA2B1	FGF4
	GARS1	SERPINA1
	SFRP4	TUBB2A
	PGAM2	LPL
	EGFR	GABRG3

	CHCHD2	CHRM1
	NCF1	IDS
	HSPB1	STAT3
	CD36	ADRA1B
	ABCB4	VAMP1
	ABCB1	VAMP2
	PON1	VEGFB
	PON2	TK1
	IBD11	ACHE
	ARPC1B	SLC5A2
	RINT1	IFNGR2
	IRF5	LPCAT1
	AD10	LPCAT2
	NOS3	HRH2
	PRKAG2	HTR2B
	AIS2	BCL2
	GATA4	DPP4
	CHDS9	PLA2G1B
	NEFL	FGF2
	AD12	CA14
	LYN	HTR2A
	CMT2H	CALCR
	JPH1	CALCRL
	GDAP1	IL6
	PMP2	EGF
	CDH17	STS
	MYMY3	GRIA1
	TNFRSF11B	MCL1
	TG	IL17RA
	NDRG1	IL17RB
	ZFAT1	IL17RC
	SLURP1	IL17RD
	AIS3	IL17RE
	AD11	NPC1L1
	CHDS8	SCN11A
	FOCAD	VDR
	VCP	PPOX
	HSCR5	CAMP
	ABCA1	CHRNA7
	KIAA0720	GLP2R
	PARK7	CACNA1A
	CMT2A	SMAD3
	KIAA0214	SLC19A1
	PARK6	NECTIN4
	HOPS	ITGAL
	HDFNRH	MC1R
	RHNA	SLCO4C1

	YARS	GLP1R
	HLD23	F2R
	AAOPD	BLVRA
	AIS1	SLC23A1
	DJC6	F5
	IBD17	CD22
	CCDD	CNR2
	ABCR	ANXA1
	GDE	RRM2
	BCATE2	CACNA1B
	CMT2DD	PTGER2
	NIID	CD33
	URBWD	CALM1
	KIAA1441	CALM2
	PUM	CALM3
	PK1	SLC47A1
	LMN1	SCN5A
	IGSF4B	NR3C1
	AILJK	IMPDH1
	CMT1B	MET
	C1orf106	XDH
	RTD	DPEP1
	CSIF	TAS1R1
	TIL3	TAS1R2
	AD4	TAS1R3
	TACE	ADRA2B
	NAG	TRPM8
	FLDB	IL1B
	MTPA	GRIA2
	MTDPS6	GABBR1
	GBD4	GABBR2
	OMI	FECH
	SRK	F7
	IL1F7	SCN10A
	HOX4D	ADRB1
	PDE11A1	CRHR1
	PPH1	PLAT
	IDDM12	CHRM2
	FARSLB	SIGMAR1
	IFI41	RXRG
	KIAA0642	TLR7
	APG16L	MTTP
	RP47	DRD5
	SPAT	SCT
	SLEB2	P2RY12
	MYMY	PPIA
	LQT9	TACR1

	GPR13	FGFR1
	GSD4	PRKCG
	ADMIO3	IL11RA
	CLP46	HPSE
	SEC61	OPRM1
	CMT2B	IGF1R
	BCPM	MTNR1B
	DZIP2	SLC18A2
	GSD15	TERT
	CD10	HBA1
	CMTD1F	HBA2
	EIF4G	KCNQ1
	HEDJ	CLCN2
	HD	GLRA1
	HLN2	ITGA2B
	PROML1	ITGB3
	PARK5	CD86
	NBPHOX	NGFR
	VAMAS5	NTRK3
	SCDR9	CACNA1C
	PP2CM	TPO
	NACP	FUT4
	ADH3	ROCK1
	AIS5	ADRB3
	KIAA0517	NR1H4
	HSCR3	GABRA2
	PPNAD3	GABRA1
	GS	TPH1
	CMRD	TPH2
	HARS	ATP4A
	LARS	SCNN1A
	KIAA1985	SCNN1B
	LRG47	SCNN1G
	P62	SCNN1D
	DDX16	ACLY
	SPDA1	MMP1
	CELIAC1	ADRB2
	CMT1J	SSTR2
	PKHD1	NAGLU
	SIASD	ANGPTL3
	ADMD	CA4
	E1B	ADRA1D
	PCLD2	PDGFRA
	KIAA0274	HMGCR
	PDNP1	LYVE1
	PARK2	CACNA1H
	SCA17	ASIC1

	IFNB2	GLRA2
	IBMPFD2	GLRA3
	GARS	GLRB
	FRPHE	CFTR
	PGAMM	LIPE
	NISBD2	CHRNA2
	PARK22	AMD1
	CGD1	SLC6A3
	HSP27	TPP1
	CHDS7	CD19
	PGY3	ALK
	PGY1	ABCG2
	PON	CXCR2
	ARC41	CYP2D6
	ILFS3	GUCY1A1
	IBD14	GUCY1B1
	WPWS	KIT
	VAMAS3	CD80
	ASD2	FGFR2
	CMT2E	PPARG
	SAIDV	OPRD1
	JP1	ABAT
	CMT4A	PDGFRB
	CMT1G	CD58
	CDH16	EGFR
	OPG	GHSR
	AITD3	PDCD1
	HMSNL	HTR4
	ZNF406	FHIT
	MDM	PPP1CA
	VAMAS4	AVPR1B
	KIAA1797	TYMP
	IBMPFD1	RXRβ
	ABC1	GCGR
	DSMA4	ODC1
	CMT2A1	CD1A
	CMT2A2A	TSHR
	TNSALP	RET
	CMTDIC	OPRK1
	VAMAS2	C5
	KIAA0473	SOST
	STGD1	AKR1C3
	HOMGSMR2	DHODH
	ETM6	REN
	PDB6	RARB
	ADTKD2	IL2
	EMD2	PDE4A

	TSL1	PSMA1
	CMTDID	PSMA2
	IBD29	SLC6A1
	ADTKD4	DDC
	GVHDS	PCSK9
	SLEB1	GRIN1
	STM2	GABRA3
	NISBD1	TH
	SOPH	GRIA3
	LDLCQ4	SYK
	CMT2EE	JAK1
	STSL1	SLC5A6
	PARK13	ALB
	ADMIO2	G6PD
	FIL1Z	ABL1
	PDE11A2	ADCY1
	POVD1	IFNA2
	CELIAC3	FLT1
	RILDBC1	CHRNA4
	IFI75	CHRNA2
	PARK11	MAP1A
	IBD10	MAP1B
	RP96	HTR3A
	MPDT	HRH3
	V28	CACNA1G
	APBD	SSTR5
	KTELC1	EPOR
	ADTKD5	GPB1
	PSN	ALDH3A2
	HHD	ADH1A
	PKD5	AVPR2
	CALLA	RAB9A
	PARK18	SMO
	DJ9	TNFRSF17
	IT15	DRD2
	AC133	CHRM3
	SPG79	PTH1R
	PMX2B	PIK3CD
	FLDP	ESRRA
	PTMP	ESRRB
	PARK1	ESRRG
	CMT2R	MMP2
	ADSD	FSHR
	FPC	SLC25A4
	SARA2	HDAC1
	USH3B	ROS1
	LFIS	HCRTR2

	MNMN	NR1I3
	IFI1	AOC3
	PDB3	GHRHR
	DBP2	SLC22A8
	ARPKD	PGF
	SLD	SNAP25
	STGD2	CYSLTR1
	SAC3	NPR1
	NPPS	IL3RA
	PDJ	SMN1
	HDL4	SMN2
	BSF2	NR3C2
	OPMD2	MC4R
	SMAD1	IL1R1
	PYL	CSF2RA
	GSD10	MS4A1
	CMT2F	RRM1
	BDPLT10	RRM2B
	MDR3	MMP7
	MDR1	SERPINC1
	ESA	GRIK1
	IMD71	SV2A
	SLEB10	FABP1
	CMH6	CSF2
	VSD1	FLT3
	CMT1F	PLAUR
	CMT2K	AGTR1
	HPT1	KCND3
	OCIF	MTOR
	TDH3	TSPO
	CMT4D	NQO2
	SCOLIV	PTGFR
	CMT2Y	CPT1B
	HPALP1	DPYD
	CMTRIC	C1S
	NBLST1	FKBP1A
	HMSN6A	TBXA2R
	HPPA	PAH
	TYRRS	CES1
	PARK19	GRIN2B
	FFM	PGD
	OPDM3	CD52
	FPLD2	CNR1
	NECL1	COMT
	DSS	GSK3A
	MELIOS	SCN3A
	CMD1V	PRDX5

	ILFS2	MT-ND3
	FCHL2	ADORA2A
	PRSS25	IFNG
	IMD48	TRHR
	IL1H4	HRH1
	PDE11A3	ASRGL1
	ALPS5	IL5
	VODI	HTR1F
	RMD2	HTR1A
	RUMI	AVPR1A
	NEP	TYMS
	ABBP2	LHCGR
	LOMARS	THRA
	RP41	MAPK12
	NDGOA	CHRM5
	NBLST2	PIK3CA
	MSUDMV	GABRB2
	PARK4	ESR2
	BTPS2	KDR
	ANDD	IDE
	CMT2W	RARG
	ILFS1	FCGR3A
	IBD19	FCGR3B
	FTDALS3	AXL
	PRP8	ACE
	PKD4	ITPR1
	STGD3	ITPR2
	ALS11	ITPR3
	M6S1	KLKB1
	HSF	CTNNB1
	CMT2D	INS
	HMN2B	MAOB
	ICP3	NFKB1
	IBD13	NFKB2
	MVCD5	RELA
	TACHD	RELB
	CMTDIG	REL
	CMTRIA	TRPV1
	PDB5	F9
	FTDALS6	FOLR1
	TGD	HTR2C
	CMT2A2B	MAOA
	HPPO	TRPA1
	YTS	HTR1E
	RP19	CACNA2D1
	CMD1A	OXTR
	SYNCAM3	TXNRD1

	CHN2	CYP11B1
	MGCA8	F10
	IL1RP1	NTRK2
	PPNAD2	SLC12A3
	C3orf9	INSR
	CMT2T	CD28
	PKD6	TOP2B
	CORD12	CDK4
	SPG79A	SRC
	CCHS	ALAS1
	DESMD	SLC22A12
	NADGP	APOC3
	NMOAS	FDPS
	ISQMR	PPARA
	YVS	ADORA2B
	PCA1	PTAFR
	HGF	APP
	HMN5A	CD3E
	CLCs	GPR55
	TOF	TNFRSF8
	HDLCQTL13	GGCX
	MSL	HCAR2
	HPPI	SERPINA6
	YRS	PDE3A
	CORD3	PLG
	HGPS	CYP17A1
	CMT2FF	IMPDH2
	IBD31	CDK6
	DDD4	PDF
	SCA43	PTPN1
	CD133	ESR1
	DMRV	IDO1
	SCA34	IL5RA
	BTOP	FGFR3
	ARHR2	ABCC8
	SMAJI	ERBB4
	HPPC	ADORA1
	IMNEPD2	CASR
	ARMD2	DPYSL2
	LGMDR21	SLC9A3
	MCDR2	EEF2K
	COLED	ADRA1A
	STGD4	ATP4B
		MGAM
		EDNRB
		SQLE
		IL6R

		IL6ST
		GPR119
		SLC12A1
		F2
		PNLIP
		AOX1
		LDLR
		IL12B
		FCER1G
		SST
		DGAT2
		PIK3CG
		CXCR4
		TOP2A
		GRIA4
		GUSB
		VEGFC
		VEGFD
		IL37
		TYR
		FOLH1
		M6PR
		TGFB1
		ITGA4
		MTNR1A
		PPAT
		GUCY2C
		SLAMF7
		ARSA
		PTH
		CSF3R
		MRM2
		THRB
		BRAF
		SLC10A1
		TAAR1
		ALDH1A1
		ALDH2
		ALDH1B1
		BCHE
		CD274
		PTGIR
		CSNK1E

3.2. PPI network analysis of key targets of the Silymarin-Pueraria lobata-Prednisolone combination for ALD treatment

The targets of Silymarin, Pueraria lobata, and Prednisolone were examined for intersections with ALD targets. The language is formal, with appropriate hedging and passive voice, and adheres to

British spelling and grammar conventions. Figure 1 illustrates the Silymarin-Pueraria lobata complex, which displays 11 intersecting targets with Prednisolone, 88 intersecting targets with ALD, and 12 intersecting targets with Prednisolone. To obtain the PPI network, the targets of Silymarin-Pueraria lobata, Prednisolone, and ALD were intersected, followed by concatenating their interactions (Fig. 2). The network consists of 95 nodes and 914 edges. The maximum degree value is 59, the minimum degree value is 1, and the average degree value is 19.2 (refer to Table 4). The nodes represent the target proteins, while the edges represent their interactions. The degree value indicates the target's importance compared to all other targets. Figure 3 displays that in the constructed Cytoscape network graph, the concentration of circles increases from the outer region towards the centre while following the descending order of target degree value. This indicates the rising level of association between ALD and the target. The network analysis identified ALB, IL6 and TNF as key target proteins based on their degree value. These molecular functions and targets are essential for the process of Silymarin-Pueraria lobata-Prednisolone combination therapy for ALD.

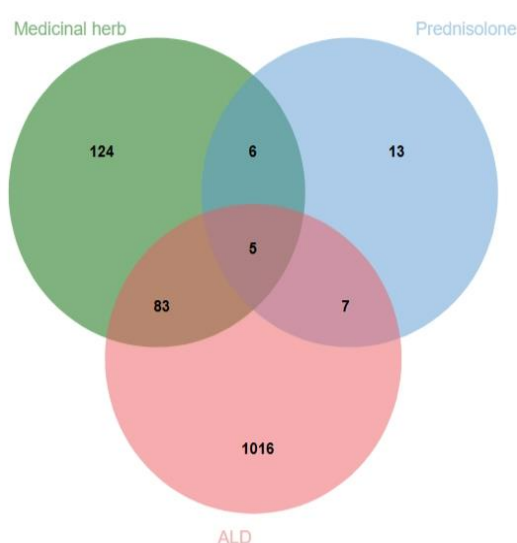


Figure 1 Silymarin-Pueraria lobata, Prednisolone, ALD target intersects

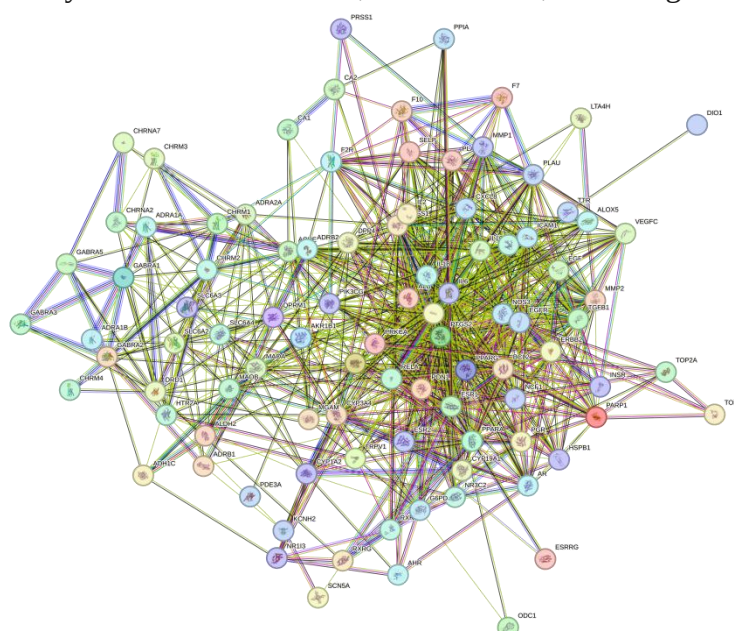


Figure 2 Silymarin-Pueraria lobata-Prednisolone Combination Therapy ALD Target PPI Interaction Networks

Table 4 PPI network related parameters

Parameter	Numerical value
number of nodes.	95
number of edges.	914
average node degree.	19.2
avg. local clustering coefficient.	0.595
expected number of edges.	291
PPI enrichment p-value.	< 1.0e-16

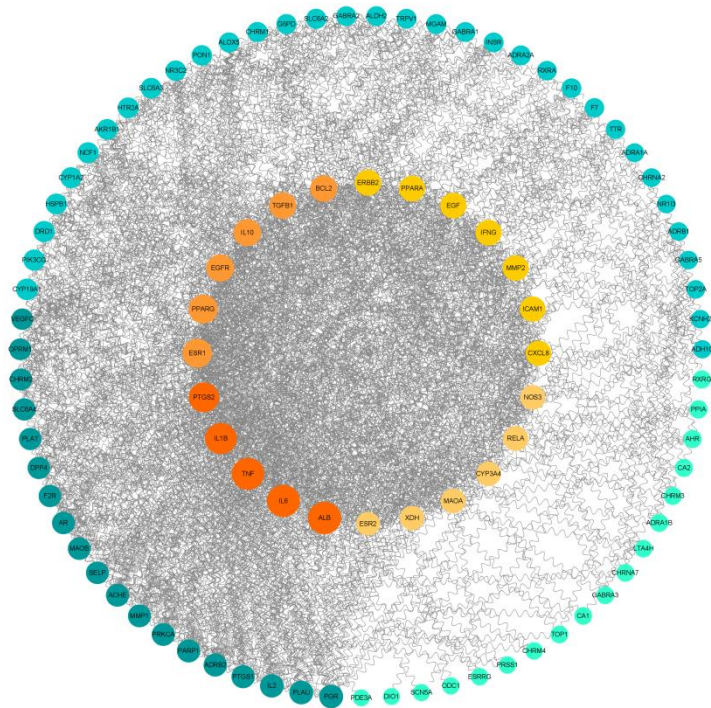


Figure 3 Network diagram of key target interactions

3.3. Results of bioinformatic analysis (GO analysis, KEGG pathway analysis)

The research found, using GO enrichment analysis, that the combination of Silymarin, Pueraria lobata, and Prednisolone impacts the biological processes, molecular functions, and cellular components of ALD. Figure 4 demonstrates that the combined intervention predominantly affects biological systems, particularly those related to blood circulation, cellular response to organic cyclic compounds, and circulatory system processes. The agent focused on multiple cellular components, such as the postsynaptic membrane, synaptic membrane, membrane raft, membrane microdomain, and receptor complex. Technical term abbreviations are clarified on their first usage. Furthermore, it specifically impacted nuclear receptor activity, ligand-activated transcription factor activity, neurotransmitter activity and complex, alongside other microscopic processes. After conducting KEGG analysis, the drug action pathways for ALD were chosen based on the P-value in the Silymarin-Pueraria Lobata-Prednisolone combination therapy pathway. The significant pathways in the combination of Chinese and Western medicine for ALD comprise of Chemical carcinogenesis - receptor activation, Neuroactive ligand-receptor interaction, Pathways in cancer, and Calcium signaling pathway, which are illustrated in Figure 5.

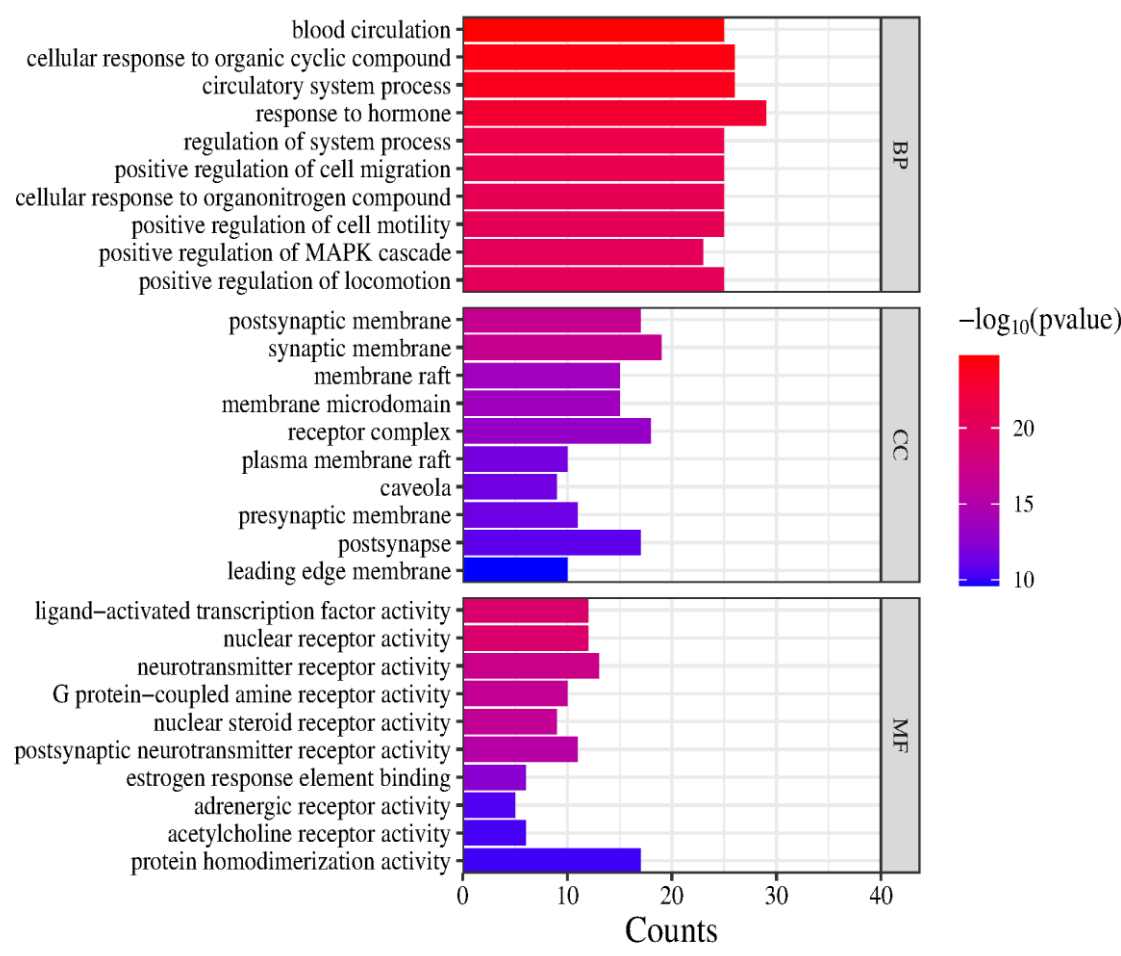


Figure 4 Silymarin-Pueraria lobata-Prednisolone Combination Therapy for ALD GO Analysis Graphs

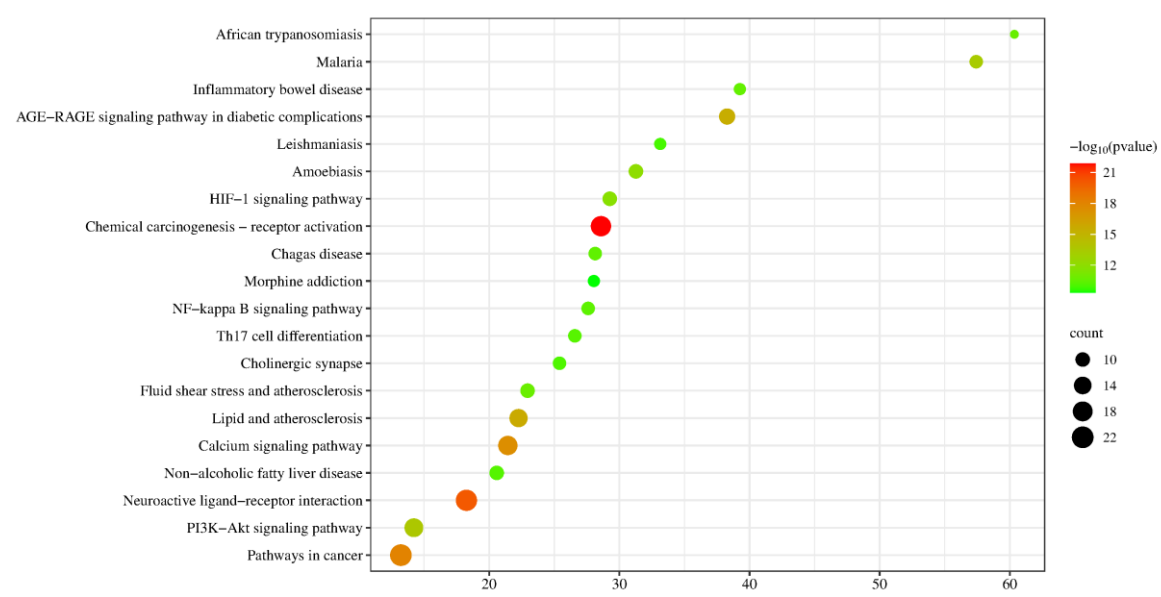


Figure 5 Silymarin-Pueraria lobata-Prednisolone combination for ALD KEGG pathway analysis

Through rigorous research and analysis of the "target-component-mechanism-pathway", a detailed understanding of the relationship between the combination of Chinese and Western medicines with the target pathway can be comprehensively explained. Additional insights into the impact of

silymarin-Pueraria lobata-prednisolone on agents such as quercetin, arachidonic acid, formononetin, beta-sitosterol, prednisolone and others can be gleaned from Figure 6 and Table 1. This combination targets ALB, IL6 and TNF. This treatment deals with prednisolone and other substances linked to chemical carcinogenesis. It covers receptor activation, the interaction between neuroactive ligands and receptors, carcinogenic pathways, the calcium signalling pathway and other targets. Moreover, calcium signalling, in conjunction with other pathways, has a considerable impact on significant biological processes at both the microscopic and macroscopic level. These pathways also have the potential to generate therapeut advantages.

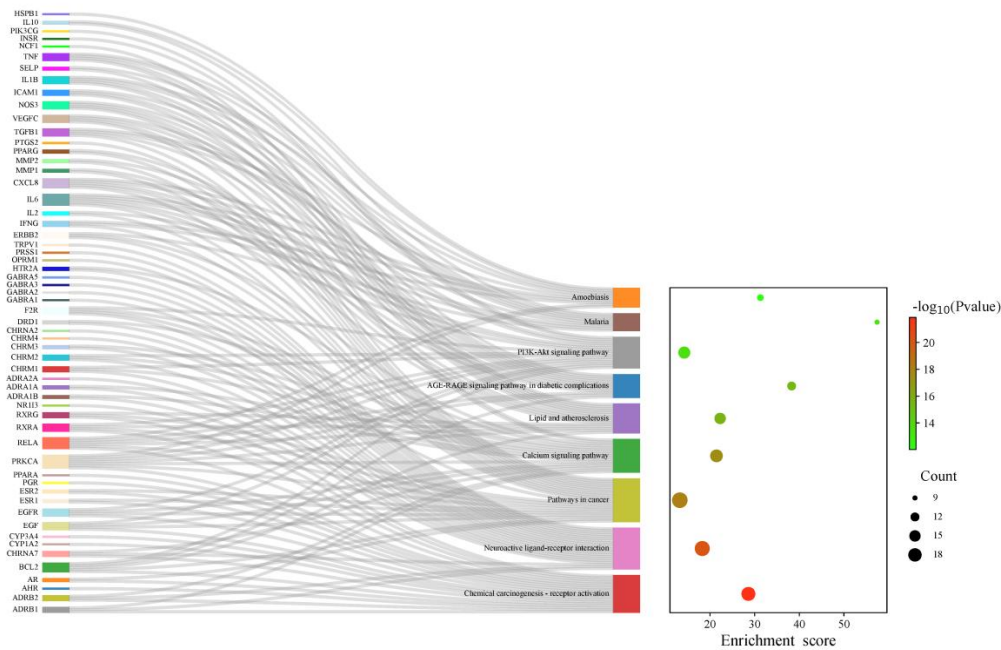


Figure 6 Silymarin-Pueraria lobata-Prednisolone combination therapy ALD target pathway Sankey-bubble plot

3.4. Validation of molecular docking of important active ingredients of drugs with key ALD targets

The molecular docking analysis was conducted on the three primary targets, namely ALB, IL6, and TNF, following their identification through screening. The Silymarin-Pueraria lobata-Prednisolone combination's primary active constituents, such as quercetin, arachidonic acid, formononetin, beta-sitosterol, and prednisolone, were subsequently categorised based on their degree values. The results, displayed in Table 5, demonstrate the diverse affinities between the primary active ingredients and their target receptors. Beta sitosterol-ALB, beta sitosterol-IL6, and quercetin-ALB are the three top compounds, with binding energies of -9.2 kJ/mol, -8.5 kJ/mol, and -8.4 kJ/mol, respectively. Further details on the active ingredients and their targets can be found in Figure 7.

Table 5 Active ingredient-target molecule docking binding. energy.

Active ingredients	Binding affinity (eV)		
	ALB	IL6	TNF
quercetin	-8.4	-7.8	-6.6
arachidonic acid	-6	-6	-4.5
formononetin	-8	-7.5	-6.4
beta-sitosterol	-9.2	-8.5	-6.5
prednisolone	-8.1	-8.2	-7.1

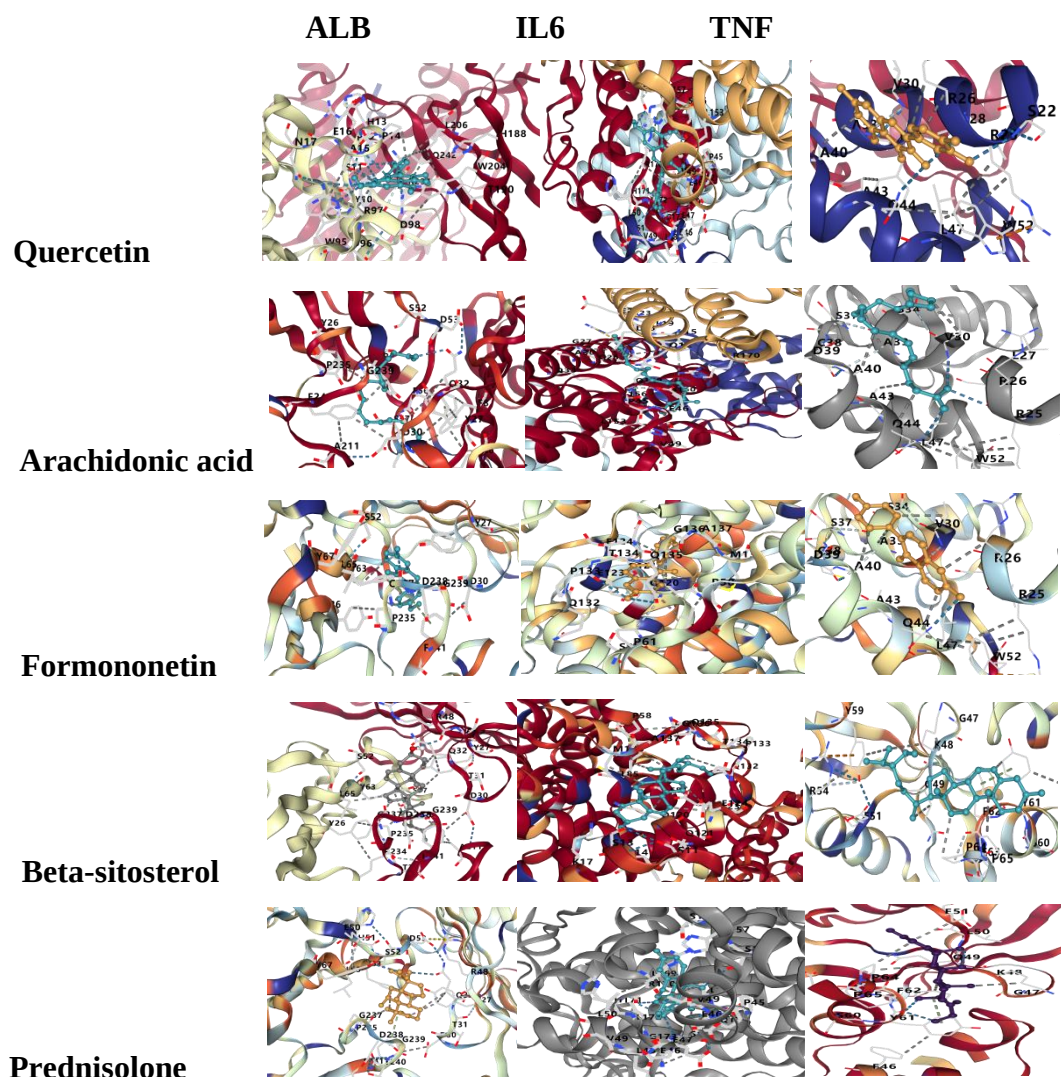


Figure 7 Schematic diagram of active ingredient-key target molecule docking

4. Discussion

The prevalence of alcohol culture has increased with the global expansion of economy and society, leading to a rise in alcoholic liver disease and its complications. In addition, numerous hazardous behaviours after drinking have emerged, which negatively affect public health and safety^[3]. The incidence of alcoholic liver disease (ALD) and its complications has increased, resulting in various unconscious hazardous conduct post alcohol ingestion that negatively impacts public wellbeing and security. Although research has made a major breakthrough in the analysis of Silymarin-Pueraria lobata combination's effectiveness in treating different stages of ALD [4], the fundamental pathogenesis of ALD remains incompletely understood. Currently, research mainly concentrates on single drug and target levels, with little examination of the mechanism of action of 'multi-target-multi-pathway'. There is limited research on the combined use of Chinese and Western medicine for treating ALD [4][5]. Also, there is a shortage of studies investigating the mechanism of action underlying 'multi-target-multi-pathway'.

In this study, we employed network pharmacology and molecular docking to screen the key targets of ALD and Silymarin-Pueraria lobata-Prednisolone. We then performed bioinformatics analysis to determine the drug's mechanisms of bioactivity during the therapeutic process. Furthermore, we verified these mechanisms through molecular docking. Technical term abbreviations are explained when first used. The drug's mechanism of action in treating ALD has been clarified, while also

providing relevant information for studying and preventing the deterioration of ALD into hepatocellular carcinoma.

The results of network pharmacology study showed that the key targets of Silymarin-Pueraria lobata-Prednisolone combination for ALD treatment are ALB, IL6, TNF. among them, ALB, as a target that has been widely used in the treatment of hepatitis cirrhosis [6], is expected to have a better role in predicting alcoholic liver disease. Studies have shown that the level of ALB is one of the risk factors for colorectal cancer prognosis[7], which also side by side confirms that factors such as intestinal microecology have an unavoidable role in the onset and deterioration of liver disease[8], and this kind of research targeting the intestinal-hepatic linkage mechanism provides mindfulness for the treatment of ALD. Xiong et al. found that, by investigating the relationship between serum IL6 and TNF with histological inflammation, IL6 and TNF, as multi-functional inflammatory factors with wide range of functions, are involved in inducing and activating tissue inflammatory response and causing tissue damage[9]. It has also been found that IL6 has a neuroprotective effect that can be exerted through the Calcium signaling pathway [10]. In addition, it has also been shown that TNF has a direct damaging effect on hepatocytes, and IL6 induces the synthesis of acute-phase response proteins in hepatocytes, and these inflammatory factors cause complications along with an irreversible vicious cycle [11]. This provides a new direction for in-depth study of IL6 and TNF in the treatment of ALD as well as exploring other unexplored mechanisms of action.

The results of the molecular docking studies suggest that quercetin, arachidonic acid, formononetin, beta-sitosterol, and prednisolone display favourable binding energy to the target molecules, rendering them critical components for the management of ALD. Hydrogen bonding predominates the binding of target proteins to small molecule active ingredients. Quercetin is highly significant in anti-tumour activity, treatment for acute kidney injury, and offers new research foundation for its chemosensitizer effect and ability to inhibit the inflammatory factor signaling pathway. This makes it a promising treatment avenue for ALD and for preventing cellular damage [12][13]. Arachidonic acid, as an endogenous active substance, can achieve an anti-inflammatory effect by promoting or inhibiting the production of its metabolites, such as PGE2 and EETs [14]. Li et al. found that arachidonic acid regulates hepatic glucose and lipid metabolism by inhibiting hepatic COX-2 activity, LOX, and CYP4A pathways, while enhancing the function of CYP2C/2J[15]. These findings provide objective evidence of metabolic pathway regulation via arachidonic acid. Clinical studies have also identified distinct anti-tumour effects from arachidonic acid and formononetin [16]. Pharmacological studies have uncovered that formononetin carries anti-inflammatory, antioxidant, anti-apoptotic, lipid-regulating, anti-thrombotic, and tumor-suppressing biological activities [17]. Furthermore, formononetin has a range of other biological activities. Additionally, research has shown that formononetin and beta-sitosterol aid in regulating inflammation and cardiovascular diseases [18][19][20]. These aforementioned studies corroborate the present findings. The aforementioned studies verify the suitability of combining Silymarin-Pueraria lobata-Prednisolone with both Chinese and Western medicine for treating ALD in this particular study. Currently, AASLD recommends prednisolone as an effective glucocorticoid drug for treating ALD in their 2010 updated guidelines for diagnosing and treating alcoholic liver disease. Prednisolone has significant anti-inflammatory, immunosuppressive, and anti-tumor properties. However, it has notable side effects, including osteoporosis, poor wound healing, muscle weakness, and developmental hindrance in embryos and young children [21]. The present study employs modern techniques to study the effects of prednisolone. In this investigation, modern technology was utilised to examine the mechanism of prednisolone in conjunction with additional traditional Chinese medicines for treating ALD. The objective was to minimise the adverse effects of prednisolone whilst promoting the application of modern pharmacological research and the integration of Chinese and Western medicinal approaches in ALD treatment.

The KEGG enrichment analysis revealed pathways of significance, including Chemical Carcinogenesis - Receptor Activation, Neuroactive Ligand-Receptor Interaction, Pathways in Cancer, and Calcium Signaling Pathway, based on P-value screening. Pan et al. discovered that prolonged

administration of diazepam on mice caused adverse effects through the interference of the Neuroactive ligand-receptor interaction pathway[22]. They provided details on the adverse effects, which shed light on their exact mechanisms. This data is vital for reducing the occurrence of adverse reactions in mice when administering medication for ALD. The calcium signaling pathway shows a strong correlation with Alzheimer's disease, cardiovascular disorders, and cerebrovascular diseases. It has been hypothesized that ALD accelerates the progression of these illnesses, although the exact mechanism is still unclear [23][24][25]. Based on the screening results, the majority of the identified pathways belong to the oncogenic pathway, indicating that a combination of Silymarin, Pueraria lobata, and Prednisolone may effectively prevent or inhibit hepatocellular carcinoma in the treatment of ALD. Further research on the disease is expected to unveil the precise mechanism of action of the target-active ingredient-pathway.

5. Conclusion

The present study examined the targets and mechanisms of action of the Silymarin-Pueraria lobata-Prednisolone combination for ALD from a 'multi-targets, multi-pathways' standpoint through network pharmacology and molecular docking analyses. The analysis revealed key targets such as ALB, IL6, and TNF, as well as relevant pathways, including Chemical carcinogenesis-receptor activation, Neuroactive ligand-receptor interaction, Pathways in cancer, and Calcium signaling. IL-6 and TNF are significant factors in various crucial pathways, including receptor activation in Chemical carcinogenesis, neuroactive ligand-receptor interaction, pathways in cancer, and calcium signaling pathways. The importance of these pathways presents a clear direction for experimental validation.

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