

Exploring the Mechanism of Dendrobine in Treating Non-Alcoholic Fatty Liver Disease Based on Network Pharmacology and Experimental Validation

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Abstract

This research delves into the therapeutic mechanisms of Dendrobine, the primary bioactive compound in *Dendrobium nobile*, for Non-Alcoholic Fatty Liver Disease (NAFLD) management. Integrating network pharmacology with experimental validation, the study evaluates the clinical effectiveness and safety of *Dendrobium nobile* in NAFLD treatment through an exploratory clinical trial. The approach identifies Dendrobine's potential targets and associated genes, constructing an interactive gene network. Validation processes include functional genomics, pathway enrichment analysis, molecular docking, cellular assays, and qPCR. Results demonstrate *Dendrobium nobile*'s efficacy in enhancing liver function among NAFLD patients. Network pharmacology findings indicate Dendrobine's influence on key targets like PPARG, IL6, TNF, IL1B, and AKT1, with molecular docking confirming interactions across these targets, excluding NFKB1. Dendrobine significantly reduced ALT and AST levels in PA-treated HepG2 cells, suggesting hepatoprotective properties, and ameliorated oxidative stress by lowering MDA levels and increasing SOD levels. The findings suggest Dendrobine's role in modulating inflammatory and immune responses, potentially through the downregulation of inflammatory mediators like TNF, IL6, and IL1B, and influencing lipid metabolism via AKT1 and STAT3 inhibition, thereby mitigating liver damage in NAFLD.

Introduction

Fatty liver disease represents a significant public health issue globally. With the rising prevalence of obesity and type 2 diabetes, the incidence of fatty liver is increasing annually. Non-alcoholic fatty liver disease (NAFLD) is one of the leading causes of liver-related diseases worldwide, posing a serious threat to public health[1]. NAFLD increases the risk of cardiovascular diseases, chronic kidney diseases, and certain extrahepatic cancers. Moreover, NAFLD and its advanced stage, non-alcoholic steatohepatitis (NASH), are major causes of cirrhosis and liver cancer. Therefore, the active treatment of NAFLD is of great importance[2].

The treatment of NAFLD currently faces many challenges. Despite numerous clinical trials over the past two decades, there are still no approved pharmacological treatments specifically for NAFLD, with few drugs successfully treating NAFLD in clinical trials[3].

In the search for effective treatments for NAFLD, *Dendrobium nobile*, a traditional Chinese medicinal herb, has gained attention due to its diverse pharmacological effects. Containing a variety of bioactive components such as polysaccharides, alkaloids, and polyphenolic compounds, it has been proven to improve metabolic disorders, reduce fat accumulation, lower inflammation levels, and improve insulin resistance, thereby holding potential value in the treatment of NAFLD.

To validate this hypothesis, we conducted an exploratory clinical trial aimed at assessing the clinical effects of *Dendrobium nobile* on patients with NAFLD.

Dendrobine, a focus of research since the 1930s, marked a significant discovery in therapeutic substances with its initial isolation and identification in *Dendrobium nobile*. Since then, dozens of alkaloid monomers have been identified from various *Dendrobium* species, among which dendrobine stands out for its significant therapeutic effects. As a class of nitrogenous organic compounds, dendrobine exhibits high biological activity and is a characteristic effective component of *Dendrobium nobile*, holding significant therapeutic value in traditional Chinese medicine. Given its significant pharmacological activity, unique biosynthetic pathways, historical significance in traditional herbal medicine, and ubiquitous presence in various medicinal *Dendrobium* species, dendrobine is considered the most important active component in *Dendrobium nobile*.

Based on this, our study focused on exploring the potential mechanisms of dendrobine intervention in NAFLD. We established a network relationship between the therapeutic targets of dendrobine and NAFLD-related target genes to elucidate the possible mechanisms by which dendrobine improves liver function.

Materials and Methods

2.1 Experimental Design

In this open-label, single-arm, non-randomized, exploratory clinical study conducted from May 2020 to May 2021, 33 patients who met the diagnostic criteria for non-alcoholic fatty liver disease (NAFLD) were enrolled from the outpatient clinic of Longhua Hospital, affiliated with Shanghai University of Traditional Chinese Medicine. The primary objective was to evaluate the safety and clinical efficacy of *Dendrobium nobile* in the treatment of NAFLD. Ethical approval was granted by the Medical Ethics Committee of Longhua Hospital (Approval No. 2020LCSY021), and informed consent was obtained from all participants. This study was registered with the Chinese Clinical Trial Registry (Trial registration: Chinese Clinical Trial Registry, ChiCTR2000034550. Date of registration: 09 July 2020. URL of trial registry record: <https://www.chictr.org.cn/showproj.html?proj=55914>), strictly following the approved protocol. The intervention consisted of oral administration of *Dendrobium nobile* granules, with each sachet containing 6 grams of dried *Dendrobium nobile* powder, administered twice daily for a continuous period of 8 weeks.

2.2 Inclusion and Exclusion Criteria

Participants were aged between 18 and 80 years; diagnosed with NAFLD via ultrasound or CT, and other chronic liver diseases were excluded. Voluntary participation in the trial was required with signed informed consent. Exclusion criteria included having cardiovascular, liver, or kidney diseases; abnormal mental consciousness; concomitant blood system diseases; pregnancy; viral hepatitis, drug-induced hepatitis, etc.; and loss of personal information data preventing statistical analysis.

2.3 Primary and Secondary Outcome Measures

The primary objective of our study was to observe changes in liver function indicators. Secondary objectives included monitoring changes in weight, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), free fatty acids (FFA), fasting blood glucose (FBG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GT). These indicators were measured at the beginning of the study and again at the conclusion of the treatment period.

2.4 Dendrobine Target Identification

To investigate the potential targets of Dendrobine, we first searched for its chemical composition on PubChem and obtained its structure in the SMILES format. This information was then uploaded to the SwissTargetPrediction[4]-[5] (<http://www.swisstargetprediction.ch/>) and PharmMapper[6] (<http://www.lilab-ecust.cn/pharmmapper/>) databases to predict the possible targets of Dendrobine. We selected 'Homo sapiens' as the screening condition to predict the therapeutic targets of Dendrobine, focusing on targets with a probability greater than zero for further analysis. Lastly, the identified targets were compared in the UniProt database (<http://www.uniprot.org/>) to determine their unique gene names.

2.5 NAFLD Target Identification

To construct a comprehensive target gene database for non-alcoholic fatty liver disease (NAFLD), we integrated relevant target genes from three distinct databases: GeneCards[7] (<https://www.genecards.org/>), DisGeNET[8] (<http://www.disgenet.org/>), and the Online Mendelian Inheritance in Man (OMIM, <https://omim.org/>). After meticulously eliminating duplicate genes, we successfully established a consolidated target gene database specifically for NAFLD.

2.6 Intersection Gene Network

Based on the databases of NAFLD-related genes and Dendrobine-associated targets, we utilized Interactive Venn[9] (<http://www.interactivenn.net/>) to identify the intersecting genes between Dendrobine and NAFLD, and created a Venn diagram to illustrate this overlap. The list of intersecting genes was exported and uploaded to the String database[10] (<http://string-db.org/>), using the 'Multiple proteins' tool and specifying 'Homo sapiens' as the species. This process generated a Protein-Protein Interaction (PPI) network for the intersecting genes, which was saved in TSV format. The TSV file was then imported into Cytoscape 3.7.2 software for network topology analysis.

2.8 GO and KEGG Pathway Enrichment Analysis

Upon identifying the intersecting targets between Dendrobine and NAFLD, we conducted Gene Ontology (GO) functional enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses for these intersecting genes using the Metascape database [11](<https://metascape.org/gp/index.html>). We applied a significance threshold of $P < 0.05$ to filter and select the enriched results.

2.9 Molecular Docking Methods

To validate the affinity of Dendrobine with core target proteins, we employed molecular docking techniques. The three-dimensional structures of the top 10 proteins from the intersecting genes were downloaded from the RCSB PDB database[12] (<https://www.rcsb.org/>), and Dendrobine's three-dimensional structure was retrieved from the PubChem database[13] (<https://pubchem.ncbi.nlm.nih.gov/>) as the ligand for docking. Using PyMOL 2.4.2 software, the receptor proteins were dehydrated. Both proteins and ligands were uploaded to AutoDock Tools to remove water molecules, add hydrogens, and calculate charges on the proteins. The docking parameters for the receptor protein were set to include the active pocket sites binding the original small molecule ligands. Finally, docking of receptor proteins with Dendrobine was performed using AutoDock Vina[14], generally considering a binding energy ≤ -5 kJ/mol as a standard for successful docking.

2.9 Experimental Validation

2.9.1 Cell Experiments

In this experiment, HepG2 cell lines, provided by the Shanghai Cell Bank of the Chinese Academy of Sciences, were cultured in DMEM medium containing 10% fetal bovine serum. HepG2 cells were incubated in medium with palmitic acid (PA) at a concentration of 1 mM/L and treated for 48 hours either with or without Dendrobine (20 µg/ml, product number: A10242-20mg, supplier: Shanghai YuanYe Biological Technology Co., Ltd). The cells were cultured in a 37°C incubator with 5% CO₂. Post-treatment, the levels of ALT and AST in cell supernatants were determined using an ELISA kit[15]. Malondialdehyde (MDA) content was measured using an MDA assay kit (product number: S0131S, supplier: Beyotime Institute of Biotechnology), and superoxide dismutase (SOD) content was assessed using an SOD assay kit[16] (product number: S0086, supplier: Beyotime Institute of Biotechnology).

2.9.2 qPCR Experiment Results

In this study, we employed real-time quantitative polymerase chain reaction (qPCR) techniques to measure the expression levels of a range of genes associated with lipid metabolism and inflammatory responses. These genes include Foxa2, G6pc, Gck, Hmgcr, Hnf4a, Igf1, Il10, Il1b, Il6, InsR, Lrs1, Ldlr, Lepr, Lpl, Mapk1, Mtor, Ndufb6, Nr1h3, Pck2, Pdk4, Pklr, Ppara, Ppard, Prkaa1, Ptpn1, Rbp4, Scd1, Slc27a5, Slc2a2, Socs3, Srebf1, Srebf2, Stat3, Tnf, Xbp1, B2m, Gusb, Hsp90ab1, MGDC, Abca1, Abcg1, Abcaca, Acox1, Acs15, Acsm3, Adipor1, Akt1, Apoe, Cd36, Cebpb, Cpt1a, Cpt2, Cyp7a1, Dgat2, Fabp1, Fabp3, Fabp5, Fas, and Fasn.

2.10 statistical analysis

Statistical analyses were conducted using SPSS 25.0 and GraphPad Prism 9.5.2 software. Continuous variables were reported as counts and means, while categorical variables were presented in frequency tables (frequency and percentage). Group differences for continuous variables were assessed using grouped t-tests or Wilcoxon rank-sum tests, and one-way ANOVA was used for multiple group comparisons. Chi-square tests or Fisher's exact tests were employed for categorical variables. Data were expressed as mean $\pm$ standard error. The relative expression of target genes in qPCR experiments was calculated using the $2^{-\Delta\Delta Ct}$ method, with experiments repeated thrice to ensure reliability. A P-value < 0.05 was considered statistically significant.

Results and Discussion

Table 1
Baseline Patient Data and Laboratory Results

Parameter	Male (N = 14)	Female (N = 19)	p.overall
Age	45.9 (10.2)	51.5 (9.95)	0.126
Height (cm)	166 (7.88)	164 (6.23)	0.484
Weight (kg)	67.9 (6.68)	66.8 (5.97)	0.635
BMI	24.6 (1.86)	24.7 (1.10)	0.859
Triglycerides (TG)	4.01 (1.79)	2.49 (1.21)	0.012
Total Cholesterol (TC)	5.21 (0.76)	5.73 (1.13)	0.127
High-Density Lipoprotein Cholesterol (HDL-C)	1.04 (0.17)	1.42 (0.34)	< 0.001
Low-Density Lipoprotein Cholesterol (LDL-C)	3.20 (0.73)	3.75 (0.81)	0.049
Free Fatty Acids (FFA)	0.55 (0.10)	0.55 (0.18)	0.872
Fasting Blood Glucose (FBG)	6.19 (1.43)	6.09 (1.89)	0.861
Alanine Aminotransferase (ALT)	66.3 (31.4)	63.0 (27.9)	0.752
Aspartate Aminotransferase (AST)	52.7 (36.1)	43.0 (14.2)	0.354
Gamma-Glutamyl Transferase (GT)	93.0 (88.7)	74.6 (63.0)	0.515
Serum Creatinine (Cre)	82.9 (11.9)	59.7 (7.39)	< 0.001

Table 2
Clinical Study Results

Parameter	Post-Treatment (N = 30)	Pre-Treatment (N = 30)	p.overall
Triglycerides (TG)	2.70 (1.18)	3.14 (1.64)	0.226
Total Cholesterol (TC)	5.44 (1.02)	5.51 (1.01)	0.784
High-Density Lipoprotein Cholesterol (HDL-C)	1.29 (0.29)	1.26 (0.34)	0.730
Low-Density Lipoprotein Cholesterol (LDL-C)	3.55 (0.77)	3.52 (0.81)	0.877
Free Fatty Acids (FFA)	0.51 (0.11)	0.55 (0.15)	0.214
Fasting Blood Glucose (FBG)	5.82 (0.87)	6.13 (1.68)	0.353
Alanine Aminotransferase (ALT)	50.1 (23.0)	64.4 (29.0)	0.030
Aspartate Aminotransferase (AST)	35.7 (13.2)	47.1 (25.8)	0.028
Gamma-Glutamyl Transferase (GT)	75.0 (57.8)	82.4 (74.3)	0.652
Serum Creatinine (Cre)	69.2 (14.9)	69.6 (15.0)	0.913

In a study involving 33 patients, 2 were lost to follow-up and 1 experienced diarrhea as an adverse reaction. Among the remaining 30 non-alcoholic fatty liver disease patients treated with *Dendrobium nobile* extract for 8 weeks, significant improvements in liver function indicators were observed. The average ALT level decreased from 64.4 U/L before treatment to 50.1 U/L after treatment, and AST levels dropped from 47.1 U/L to 35.1 U/L, with $p < 0.05$, indicating statistical significance. Lipid-related indicators also showed positive changes: average total cholesterol levels decreased from 5.51 mg/dL to 5.44 mg/dL, triglycerides from 3.14 mg/dL to 2.70 mg/dL, and gamma-glutamyl transferase (GT) from 82.4 mg/dL to 75 mg/dL. However, these changes were not statistically significant. No serious adverse events were reported.

3.2 Network Pharmacology Study Results

3.2.1 Determination of Dendrobine Targets

Due to the limited data on dendrobine targets in existing databases and literature, we predicted the targets of dendrobine through literature review and databases such as Swisspreidct and Pharm. After collating and eliminating duplicate genes, a total of 197 target genes for dendrobine were identified.

3.2.2 Determination of NAFLD Targets

We integrated relevant target genes from three databases (GeneCards, Disgent, OMIM). After removing duplicates, we identified a total of 2317 NAFLD targets.

3.2.3 Establishment of Intersection Genes

Using (<http://www.interactivenn.net/>), we established the intersection genes between dendrobine and NAFLD and created a Venn diagram, identifying 97 intersecting targets. Refer to Fig. 1A.

3.2.4 Construction of PPI Network

The list of intersecting genes was uploaded to the String database to construct a Protein-Protein Interaction (PPI) network. This network contains 94 nodes (proteins), with 1274 edges, an average node degree of 27.1, an average

local clustering coefficient of 0.681, and a PPI network enrichment p-value < 1.0e-16. Refer to Figs. 1B and 2A.

3.2.5 Compound-Target-Disease Network

Using Cytoscape 3.7.2, we constructed a network between the compound (dendrobine), targets, pathways, and NAFLD. Refer to Fig. 2C. The network displays various targets and pathways involved in dendrobine and NAFLD, along with their complex interactions.

3.2.6 Identification of Core Targets and Molecular Docking Results

The top 10 core target genes identified using the Cytohub plugin in Cytoscape 3.7.2 are PPARG, IL6, TNF, IL1B, PPARGC1A, AKT1, STAT3, NFKB1, CASP3, and BCL2. Specific information can be found in Table 3 and Fig. 2B.

genesymbol	FullProteinNames	pdbid	ligandname	Chains	bindingenergy
PPARG	Peroxisomeproliferator-activatedreceptorgamma	1I7I	AZ2	A	-6.9
IL6	Interleukin-6	1ALU	TLA	A	-5.7
TNF	Tumornecrosisfactor	6O0Y	A7M	C	-7.7
IL1B	Interleukin-1beta	8C3U	T9C	A	-6.3
PPARGC1A	Peroxisomeproliferator-activatedreceptorgammacoactivator1-alpha	3U9Q	DKA	A	-6.7
AKT1	RAC-alphaserine/threonine-proteinkinase	1H10	4IP	A	-6.1
STAT3	Signaltransducerandactivatoroftranscription3	6NJS	KQV	A	-6.2
NFKB1	NuclearfactorNF-kappa-Bp105subunit	/	/	/	
CASP3	Caspase-3	1NMS	16I	A	-6.7
BCL2	ApoptosisregulatorBcl-2	4LXD	1XV	A	-6.7

Molecular docking studies revealed that Dendrobine exhibits strong binding affinity with proteins including PPARG, IL6, TNF, IL1B, PPARGC1A, AKT1, STAT3, CASP3, and BCL2, with binding energies all below - 5, indicating effective docking interactions with these targets. Notably, NFKB1, being a nuclear factor, was not amenable to direct docking, and hence was not included in the docking analysis. The detailed results of these molecular interactions are depicted in Figs. 3, 4, and 5.

3.2.7 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) Enrichment Analysis

From the 97 intersecting genes, a total of 1404 related Gene Ontology (GO) functional enrichment terms and 153 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were identified ($P \leq 0.01$). The GO terms included 1244 biological processes (BP), 103 molecular functions (MF), and 57 cellular components (CC). The top 20 enriched terms in each category of BP, MF, and CC are depicted in Fig. 9.

The Gene Ontology (GO) analysis revealed that the enriched functions of the intersecting genes covered key areas such as the regulation of small molecule metabolic processes, response to hormones and nutritional levels, lipid storage and homeostasis, and glucose control. These functional processes are directly related to the main characteristics of Non-Alcoholic Fatty Liver Disease (NAFLD), such as lipid metabolic disorder and insulin resistance.

For instance, the regulation of hormone response and glucose homeostasis may be linked to alterations in insulin signaling pathways during the pathogenesis of NAFLD.

In terms of cellular components, the intersecting genes were primarily localized to specific subcellular structures, such as secretory granule lumen, plasma lipoprotein particle, and membrane raft. These components are crucial for intracellular signaling, substance transport, and metabolic processes. Changes in the secretory granule lumen, for example, may affect the secretion of hormones and other signaling molecules, thereby regulating the metabolic state of cells.

Regarding molecular functions, activities expressed by the intersecting genes, such as nuclear receptor activity, lipid transport, and oxidoreductase activity, are vital for maintaining intracellular environmental stability and metabolic balance.

KEGG pathway analysis showed significant enrichment of pathways related to insulin resistance (hsa04931) and non-alcoholic fatty liver disease (hsa04932), revealing the direct role of these genes in regulating key processes of glucose and lipid metabolic disorders, consistent with the core characteristics of NAFLD. Pathway enrichment related to lipid and atherosclerosis (hsa05417) suggests that the intersecting genes might be involved in lipid deposition and inflammatory processes in arterial walls, potentially revealing one of the pathways through which NAFLD may progress to non-alcoholic steatohepatitis (NASH) or even cirrhosis. The significant enrichment of the PPAR signaling pathway (hsa03320) also indicates its central role in regulating fatty acid storage and glucose metabolism, crucial for maintaining energy balance and metabolic health.

The significance of insulin (hsa04910) and glucagon (hsa04922) signaling pathways underscores their central role in glucose level regulation and response to energy states, suggesting that alterations in these pathways could be a key factor in the development of NAFLD.

3.2.8 Experimental Validation Results

3.2.8.1 Cell Experiments

The intervention with Dendrobine significantly reduced the levels of ALT and AST in the supernatant of HepG2 cells treated with palmitic acid (PA), indicating its hepatoprotective properties. Compared to the PA model group, the MDA levels in the PA + Dendrobine group decreased from 45 nM/mL to 20 nM/mL, and SOD activity increased from 150 U/L to 250 U/L. This suggests that Dendrobine effectively mitigates PA-induced oxidative stress. For details, see Fig. 6.

3.2.8.2 QPCR Results

The results showed that the relative expression levels of IL6, TNF, IL1B, AKT1, and STAT3 were significantly reduced in the PA + Dendrobine treatment group compared to the PA treatment group. This indicates that Dendrobine may exert its effects by regulating the expression of these genes. For more details, see Figs. 7 and 8.

Discussion

In our study, we preliminarily confirmed the effectiveness of *Dendrobium nobile* in improving liver function in non-alcoholic fatty liver disease (NAFLD) patients and focused on exploring the potential action mechanisms of its main active component, dendrobine. Network pharmacology results indicated that dendrobine mainly exerts its therapeutic

effects on NAFLD by regulating key targets including PPARG, IL6, TNF, IL1B, PPARGC1A, AKT1, STAT3, NFKB1, CASP3, and BCL2. Molecular docking studies further affirmed the interaction between these targets and dendrobine.

In cell experiments, dendrobine significantly lowered ALT and AST levels in PA-treated HepG2 cells, indicating its hepatoprotective role. Additionally, dendrobine mitigated oxidative stress, evidenced by decreased MDA levels and increased SOD levels, further confirming its antioxidative properties.

NAFLD, a liver disease closely related to metabolic disorder, involves various inflammatory cytokines and signaling pathways. IL-1 β , IL-6, and TNF- α play pivotal roles in its development[17]. Elevated TNF- α levels are associated with NAFLD severity. TNF- α levels in NAFLD, non-alcoholic fatty liver (NAFL), and non-alcoholic steatohepatitis (NASH) patients are higher compared to controls, with NASH patients showing even higher levels. Despite heterogeneity among studies, these findings suggest a significant role of TNF- α in NAFLD development and severity[18].

IL6 levels are elevated in NAFLD patients, suggesting a pro-inflammatory role in the disease's pathology[19]. IL1B, a pro-inflammatory cytokine, plays a crucial role in obesity-induced NAFLD, participating in inflammation induction and cytokine production, central to NAFLD pathology. The IL-1 family cytokines, especially IL-1 β , are key mediators in the progression of NAFLD to its more severe form, NASH. IL-1 β leads to hepatic triglyceride accumulation (steatosis) and is associated with increased pro-inflammatory cytokine expression in NAFLD[20].

AKT1 is implicated in metabolic dysfunction-related conditions, including obesity, metabolic syndrome, and NAFLD[21]. It plays a significant role in regulating glucose metabolism and fatty acid synthesis. Its downregulation could reduce fat accumulation and inflammation in the liver, thereby improving liver function[22]-[23]. QPCR results confirmed that dendrobine effectively reduces the elevated expression of AKT1 induced by PA. Hence, we speculate that dendrobine may alleviate liver fat accumulation and inflammation by directly or indirectly inhibiting AKT1 expression, improving liver damage in NAFLD patients.

STAT3 (Signal Transducer and Activator of Transcription 3) is a transcription factor affecting liver metabolism through promoting hepatocyte survival and differentiation. In vivo studies using high-fat diet mouse models show increased hepatic lipid accumulation and elevated STAT3 phosphorylation, while inhibiting STAT3 expression significantly reduces lipid accumulation induced by palmitic acid[24]. This parallels our cell experiment findings.

Combining clinical experiment, network pharmacology, and experimental validation results, we hypothesize that dendrobine affects these core genes, thereby improving NAFLD. It modulates inflammatory response pathways, likely directly or indirectly inhibiting the expression of genes like IL6, TNF, IL1B, and regulating inflammatory responses. This could be associated with biological processes like "inflammatory response regulation" and "cytokine stimulus response." By reducing the production and release of inflammatory factors, dendrobine improves hepatic inflammatory injury. Additionally, dendrobine might affect cell signal transduction by influencing genes like AKT1, STAT3, potentially related to KEGG pathways such as "insulin resistance," "PPAR signaling pathway," and "insulin signaling pathway." Through these pathways, dendrobine could affect liver metabolic function, reducing hepatic cell lipid accumulation and thereby improving liver injury in NAFLD patients.

Conclusion

In summary, the results of this study suggest that *Dendrobium nobile* can improve liver damage in patients with non-alcoholic fatty liver disease (NAFLD). Dendrobine, as a major characteristic active component of *Dendrobium nobile*, appears to be significantly involved in processes related to inflammatory factors and immune responses. It may directly or indirectly inhibit the expression of inflammatory cytokines such as TNF, IL6, and IL1B, thereby alleviating

liver inflammatory injury. Additionally, dendrobine may improve liver damage in NAFLD patients by inhibiting the expression of genes like AKT1 and STAT3, thus reducing hepatic lipid accumulation. However, it must be acknowledged that our clinical trials are still exploratory and lack high-quality clinical data, and the experimental validation lacks further evidential support.

Declarations

Data Availability Statement:

Materials and data pertinent to this study are available upon request via email to the corresponding author.

Conflict of Interest:

The authors declare no conflict of interest in the publication of this paper.

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Supplementary Description:

Supplementary figures related to this study are included in the supplementary materials accompanying this paper.

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Figures

[illegible]

A. Venn diagram showing the overlap of target genes: 197 targets for Dendrobine and 2317 for NAFLD, with 97 common targets between the two datasets.

B. A complex protein-protein interaction network constructed using the String website, where nodes represent different targets and lines indicate interactions or associations between them.

Figure 2

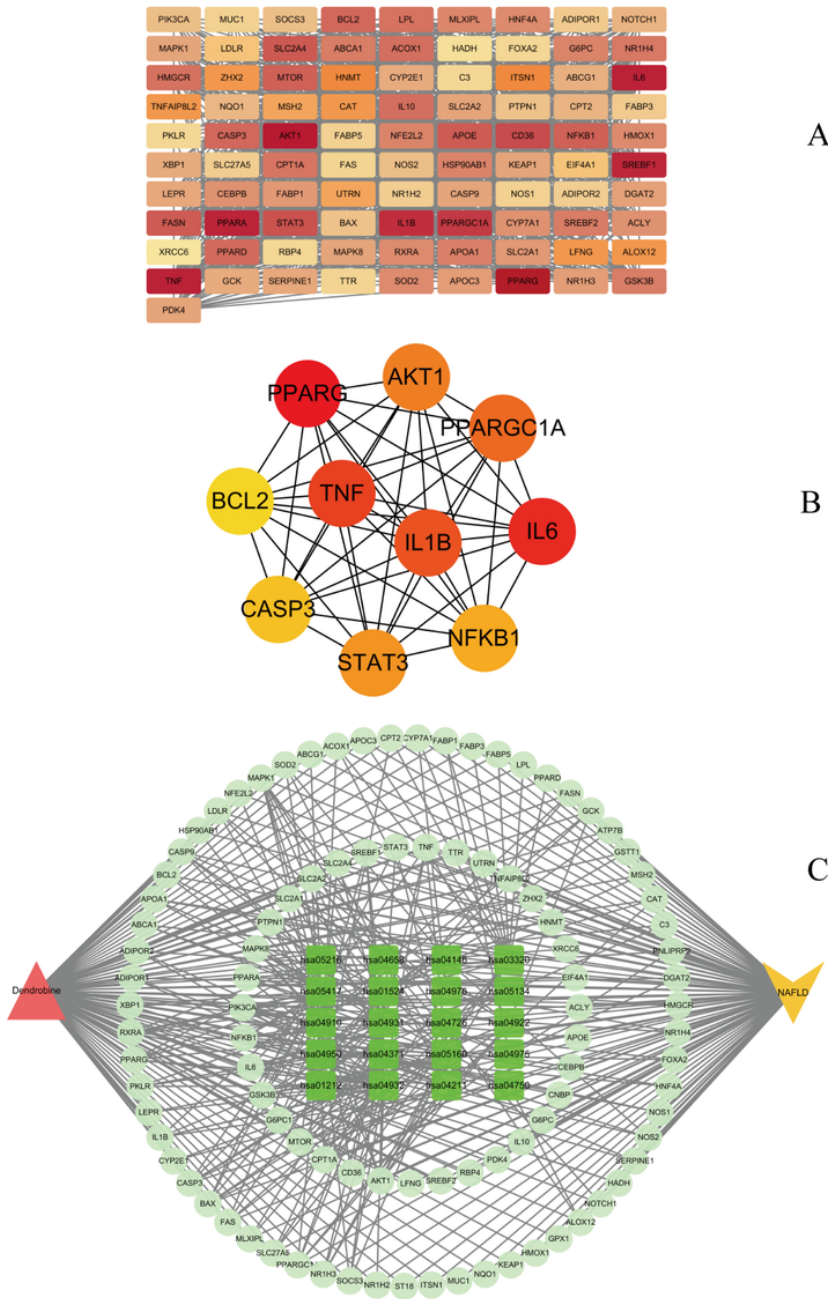


Figure 2

- A. Protein interaction network constructed in Cytoscape 3.7.2 for intersecting genes, with each rectangular block representing a target and the color intensity indicating the degree value. Deeper red indicates a higher degree value.
- B. Top 10 core targets identified using the Cytohub plugin.
- C. Network diagram depicting drug-target-component-pathway interactions.

Figure 3

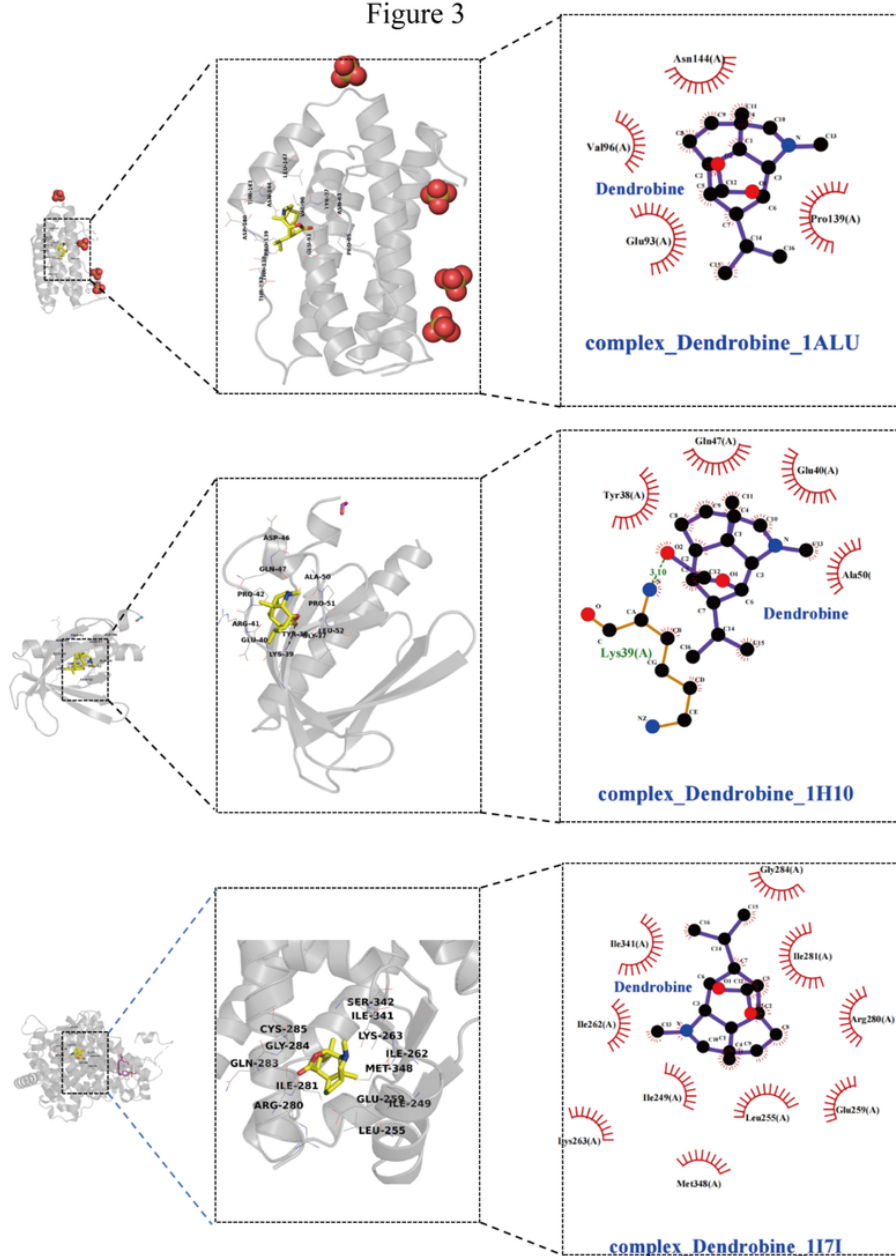


Figure 3

Illustrate 2D and 3D docking structures of Dendrobine with the top 10 core targets (excluding NFKB1). The diagrams detail specific bonding of Dendrobine with surrounding amino acid residues, represented through hydrogen bonds, hydrophobic interactions, etc.

Figure 4

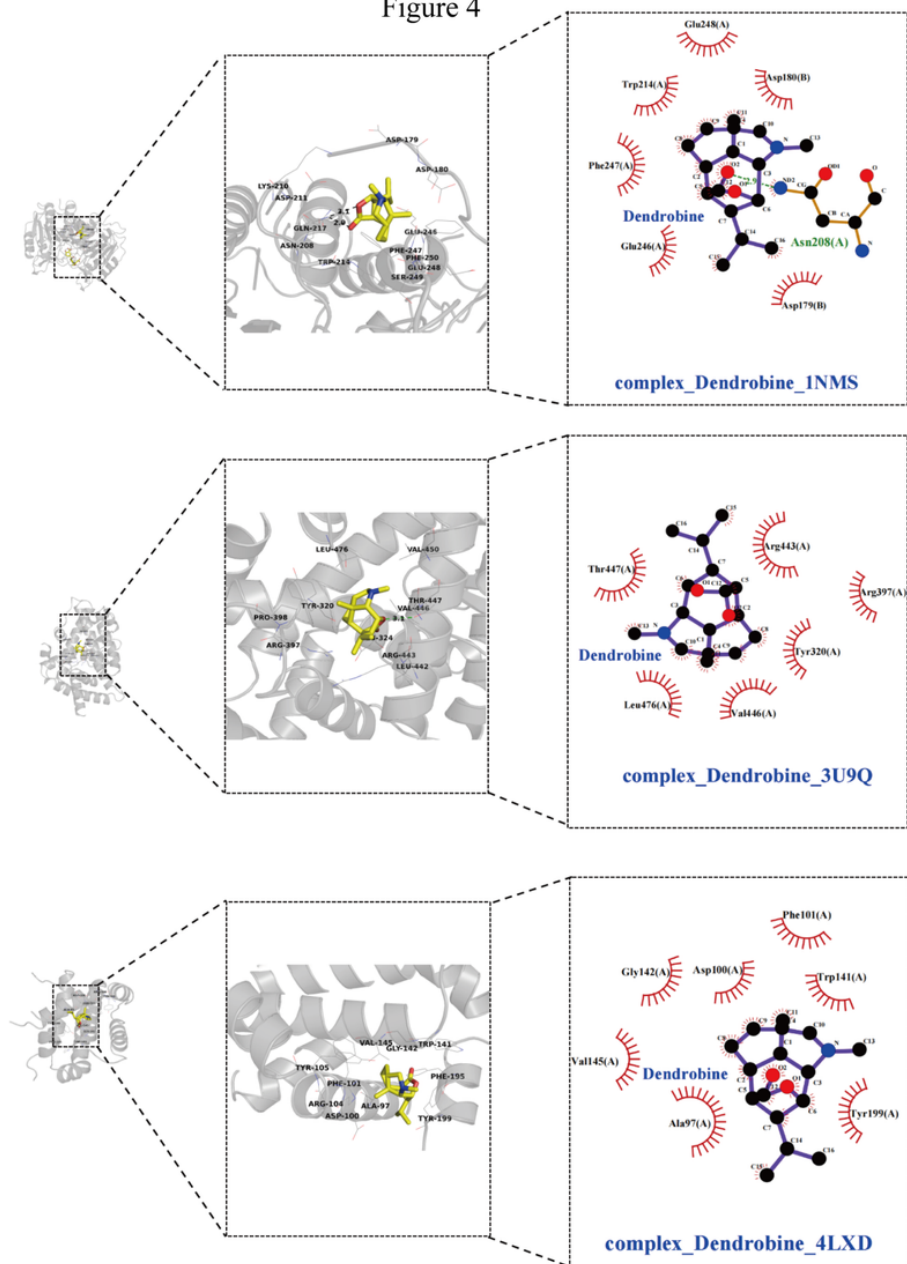


Figure 4

Illustrate 2D and 3D docking structures of Dendrobine with the top 10 core targets (excluding NFKB1). The diagrams detail specific bonding of Dendrobine with surrounding amino acid residues, represented through hydrogen bonds, hydrophobic interactions, etc.

Figure 5

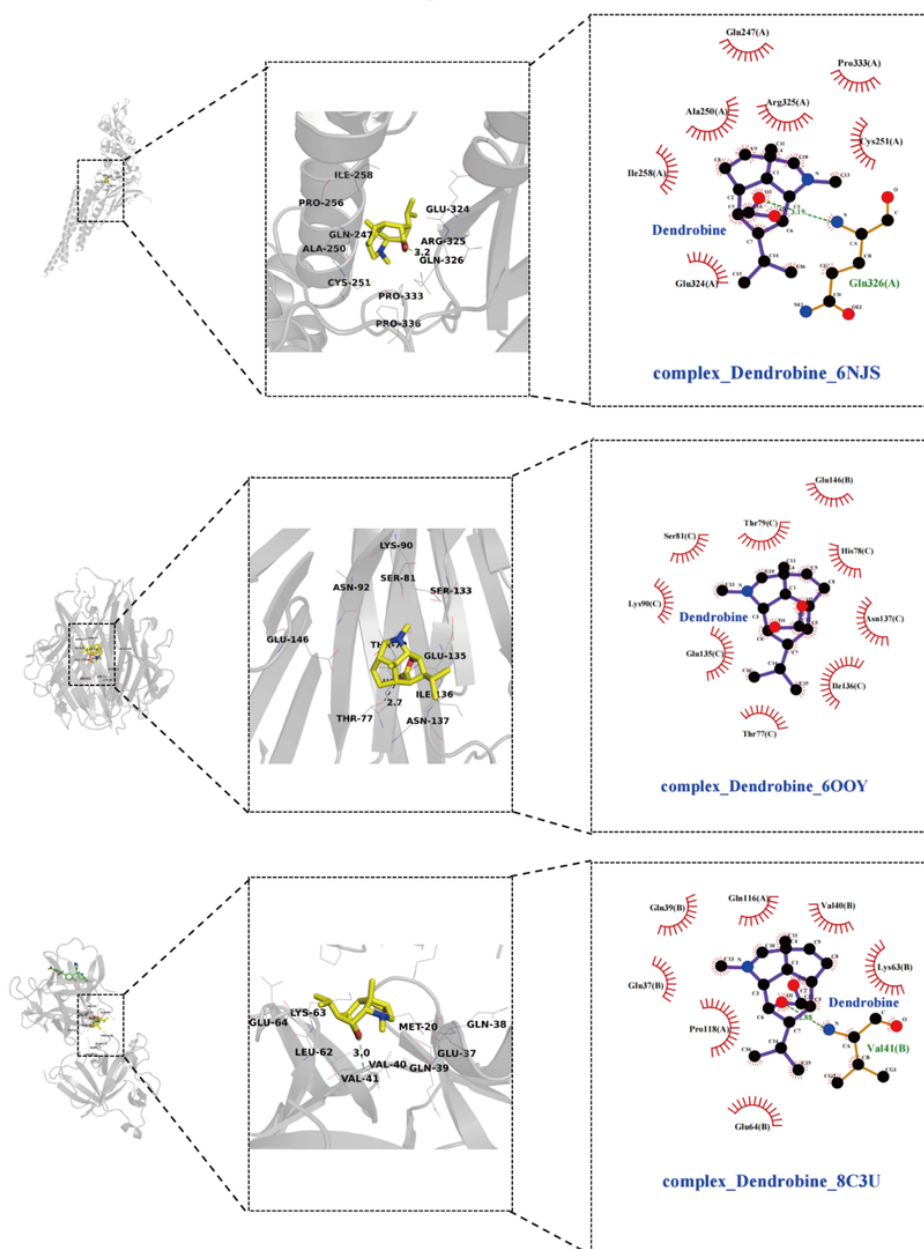


Figure 5

Illustrate 2D and 3D docking structures of Dendrobine with the top 10 core targets (excluding NFKB1). The diagrams detail specific bonding of Dendrobine with surrounding amino acid residues, represented through hydrogen bonds, hydrophobic interactions, etc.

Figure 6

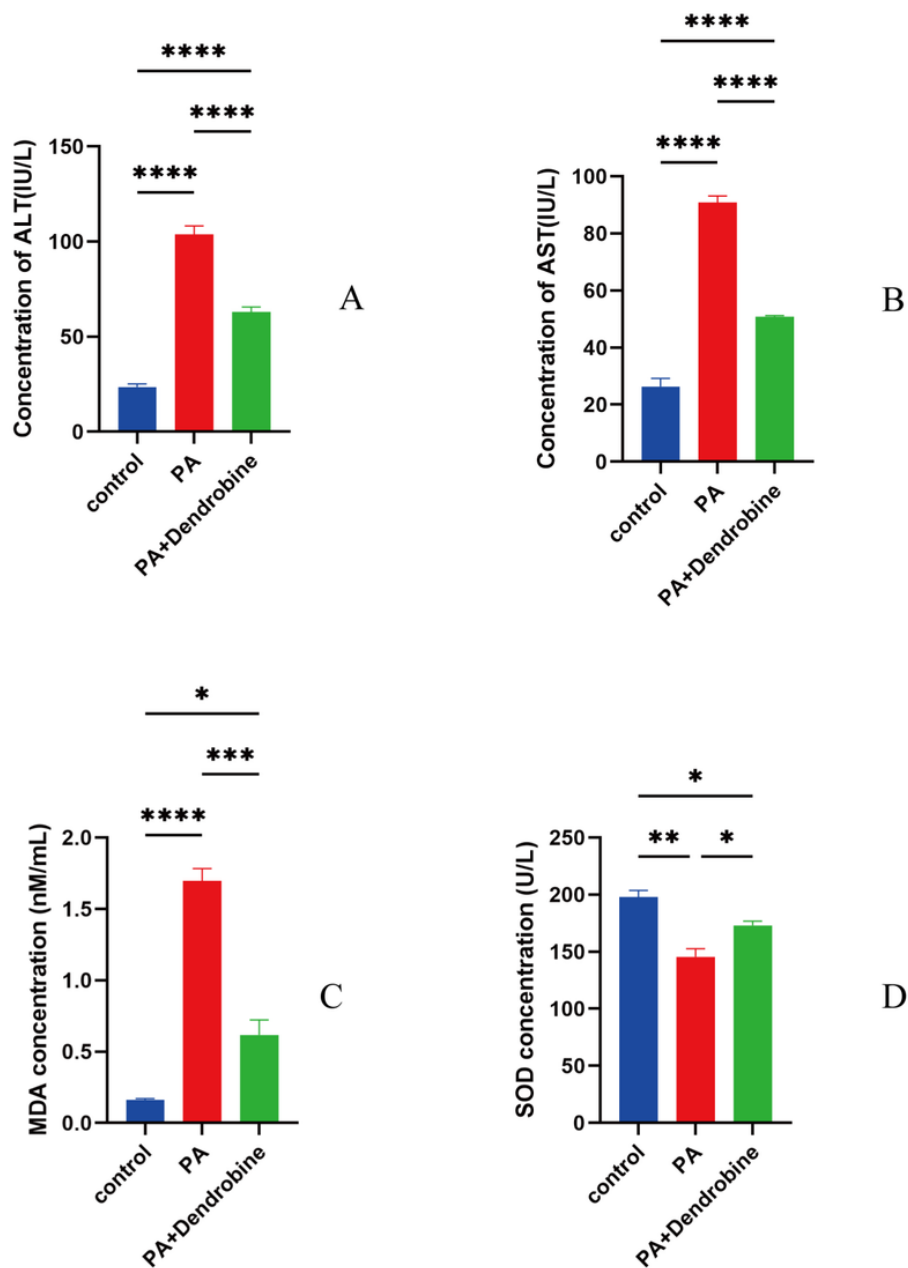


Figure 6

A-D: Concentrations of ALT, AST, MDA, and SOD under three different treatment conditions: control group (blue), PA-treated group (red), PA plus Dendrobine-treated group (green). Asterisks indicate statistical significance (*P < 0.05, **P < 0.01, ***P < 0.001). MDA levels in cell supernatants (nM/mL) reflect lipid peroxidation, while SOD activity (U/L) indicates cellular antioxidative enzyme defense.

Figure 7

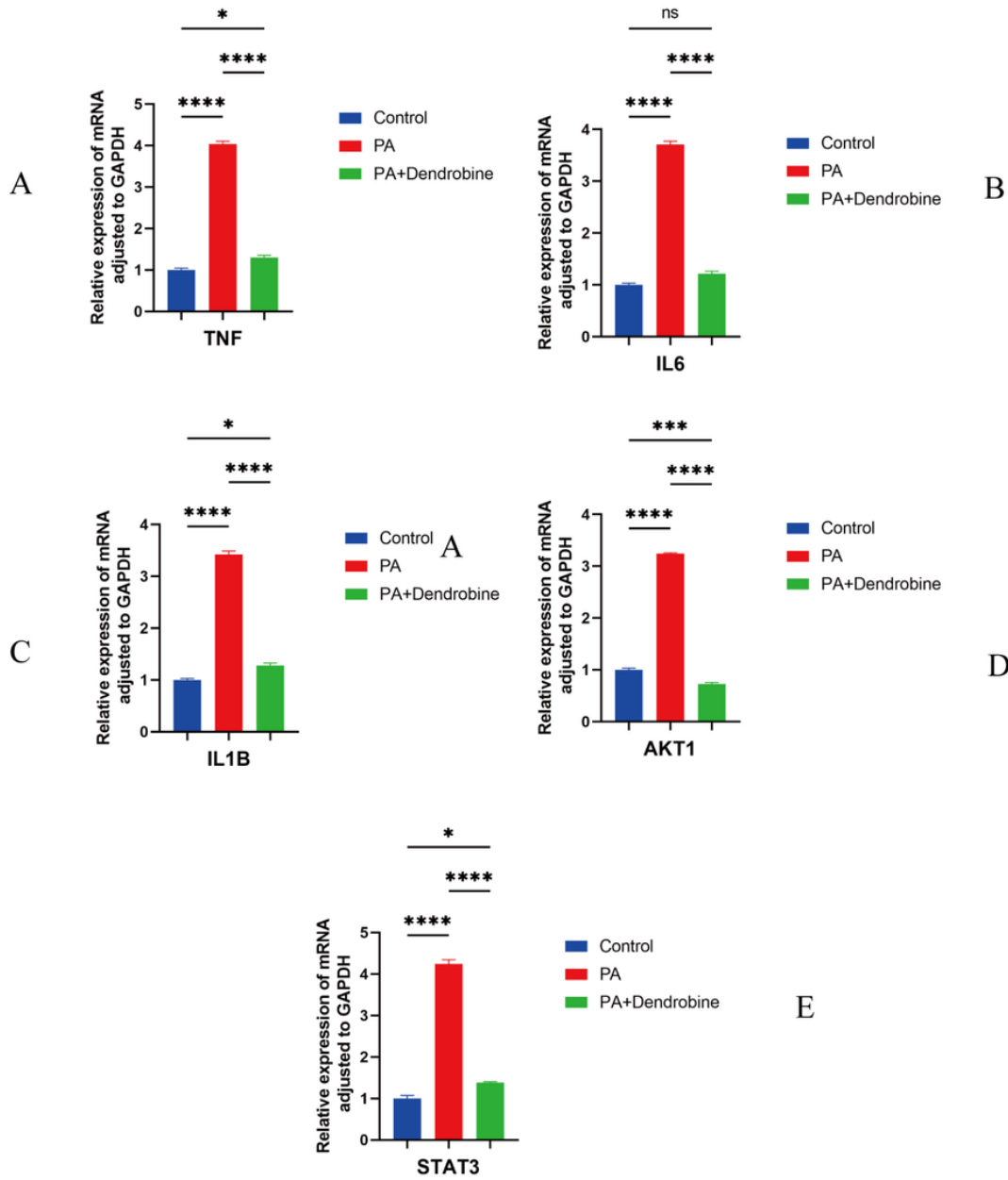


Figure 7

Shows relative expression levels of various proteins corrected for GAPDH under three different treatment conditions.

Figure 8

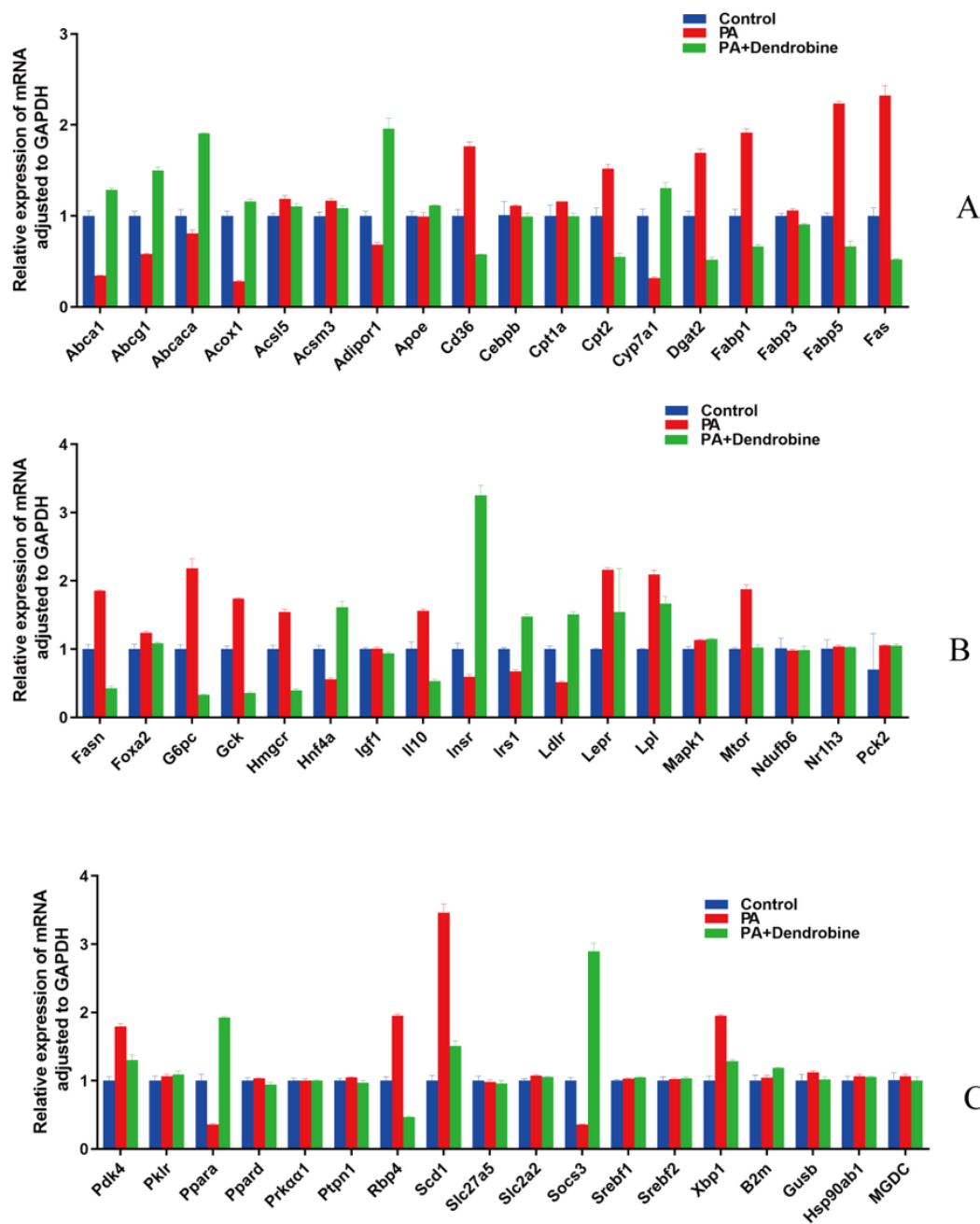


Figure 8

Displays the relative expression levels of proteins other than IL6, TNF, IL1B, AKT1, and STAT3, corrected for GAPDH under different treatment conditions.

Figure 9

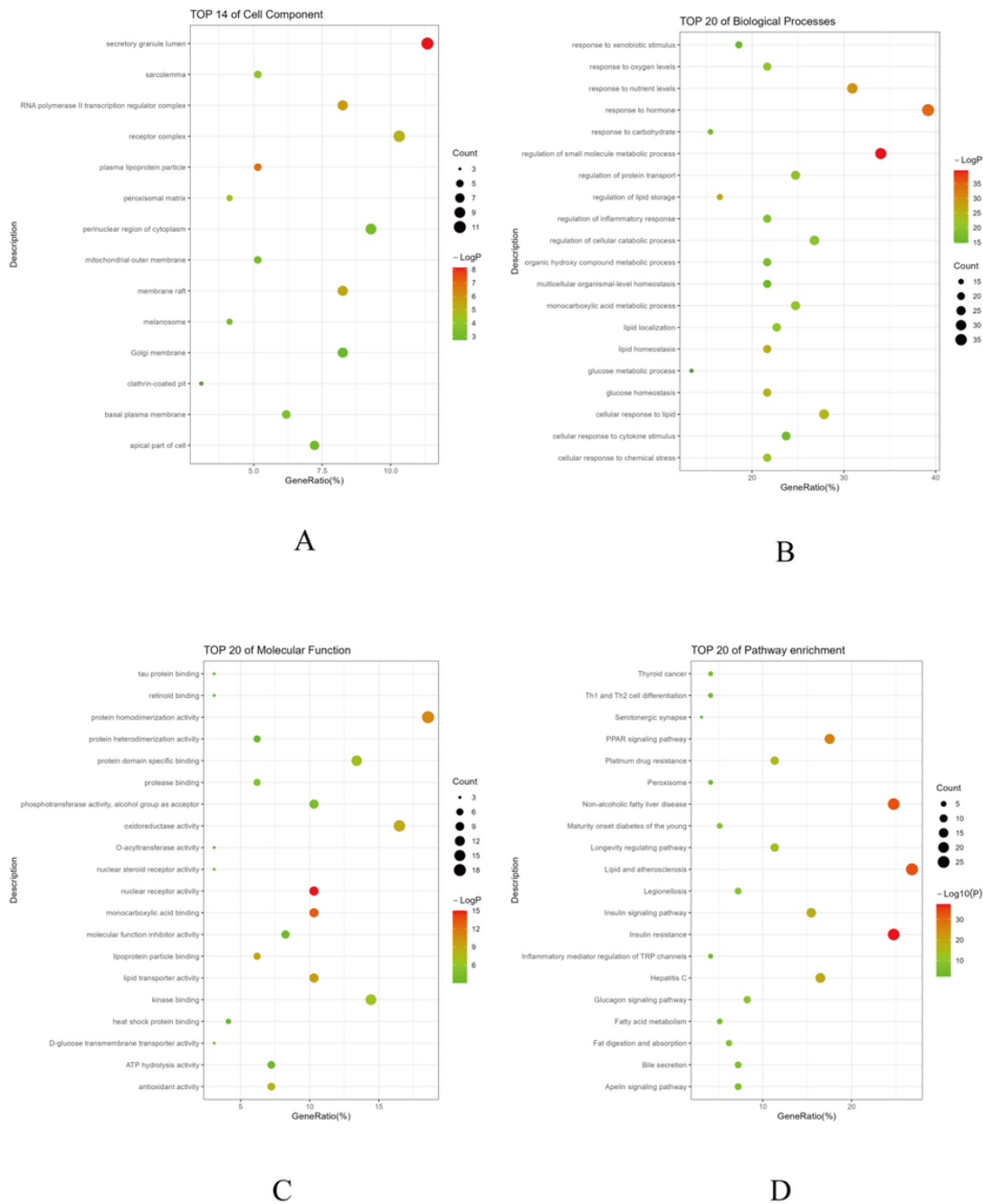


Figure 9

Presents specific results of GO function analysis and KEGG pathway enrichment for intersecting genes. Bubble size represents the number of enriched genes in each category, with color intensity indicating the significance of the P-value. Parts A, B, C, and D correspond to cellular components, biological processes, molecular functions, and KEGG enrichment pathways, respectively.