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Exploring the neuroprotective mechanisms of Jiawei Suanzaoren decoction in depression: insights from network pharmacology and molecular docking

Ruiting Ma[1†], Wenjing Zhang[2†], Lixia Chen[2*] and Lijun Tong[2*]

Abstract

Background Depression is a prevalent and debilitating neuropsychiatric disorder, often associated with neuroinflammation, oxidative stress, and neuronal apoptosis. Jiawei Suanzaoren (JWSZR), a traditional Chinese medicine (TCM) formulation, has demonstrated potential in alleviating depressive symptoms. However, its precise molecular mechanisms remain unclear. This study aims to elucidate the neuroprotective effects of JWSZR in depression using network pharmacology, molecular docking, and in vitro experimental validation.

Methods Active compounds of JWSZR were identified using the TCMSP and HERB databases, and depression-related targets were retrieved from GeneCards, DisGeNET, and OMIM. A protein–protein interaction (PPI) network was constructed, followed by functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Molecular docking was employed to predict the interactions between JWSZR's active components and key target proteins. Furthermore, in vitro experiments were performed using corticosterone (CORT)-induced PC12 cell model to validate the neuroprotective effects of JWSZR, assessing cell viability and apoptosis rates.

Results Network pharmacology and molecular docking revealed that JWSZR exerts neuroprotective effects through multiple targets, including estrogen receptor ESR2, HSP90AA1, and STAT1. These targets regulate immune responses, inflammatory pathways, and cell survival. In vitro, JWSZR significantly improved cell viability and reduced apoptosis in CORT-treated PC12 cells, indicating its potential to protect against depression-related neurodegeneration.

Conclusion This study provides novel insights into the neuroprotective mechanisms of JWSZR in depression, suggesting that it may act through multi-target interactions involving immune modulation and apoptosis inhibition.

Keywords Jiawei Suanzaoren, Depression, Network pharmacology, Protein–protein interaction, Molecular docking

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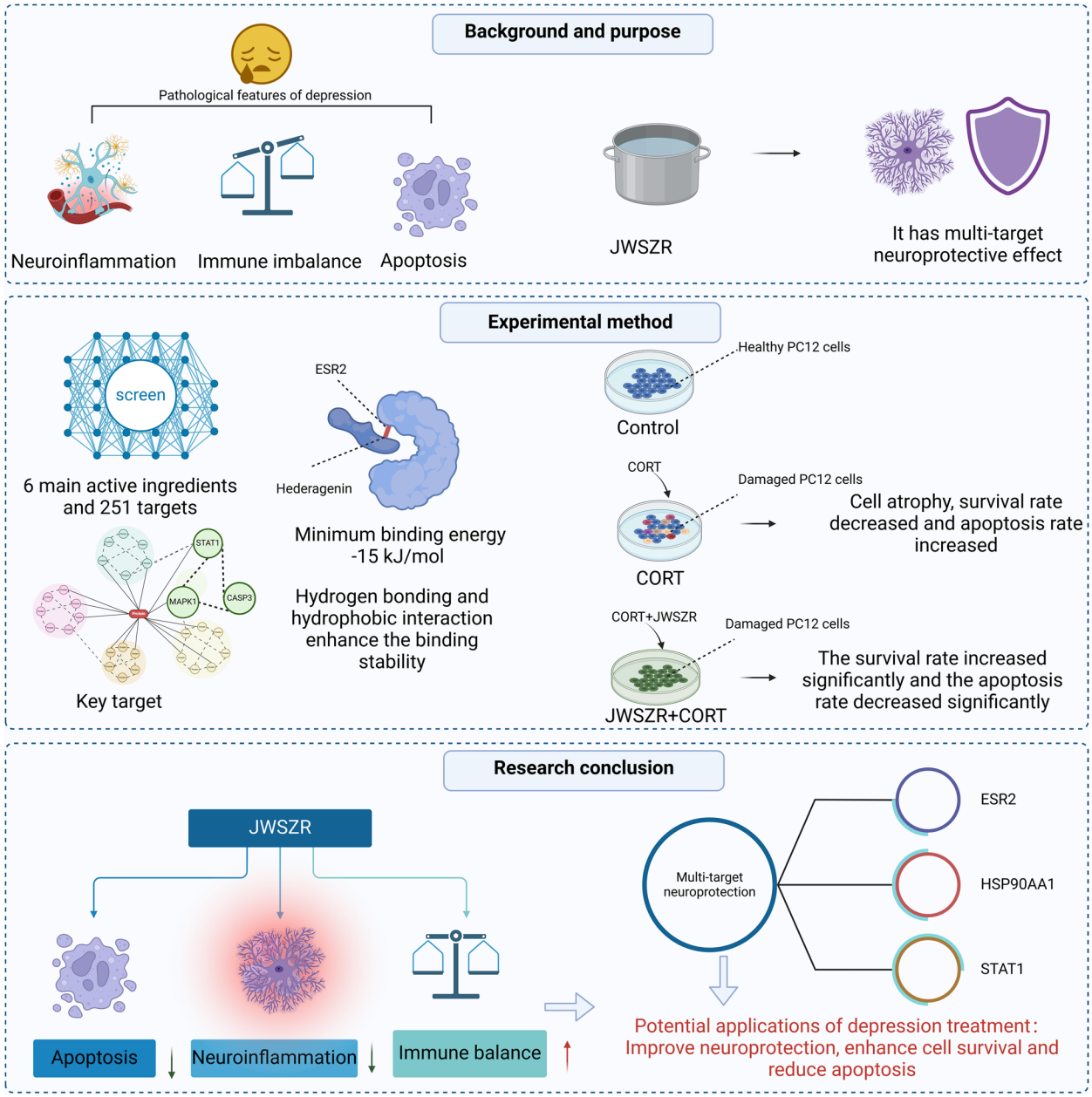
Graphical Abstract

Multi-target neuroprotective mechanism and therapeutic potential of JWSZR in depression



Introduction

Depression, a common neuropsychiatric disorder, has seen a steady rise in incidence worldwide in recent years [1], significantly impacting patients'mental health and quality of life while imposing a substantial societal and economic burden. Studies show that depression has a high global prevalence [2] and is a leading cause of disability, with a prevalence of 7%–21% [3]. Exploring effective treatments is crucial to reducing its global burden. The pathogenesis of depression is complex, encompassing immune regulation, neuroinflammation, neurotransmitter imbalance, and apoptosis [4, 5]. Although traditional pharmacological treatments, such as selective serotonin reuptake inhibitors (SSRIs) [5], are

effective, they are often associated with high rates of side effects and relapse [6, 7].

Ketamine is a rapid-acting antidepressant, but its dissociative side effects, cardiovascular risks, and abuse potential limit its use [8]. It exerts antidepressant effects via PI3 K/Akt/mTOR pathway activation, which may also accelerate brain aging, posing risks for patients with depression and aging-related diseases [9]. Additionally, depression is prevalent in breast cancer patients and is associated with recurrence and all-cause mortality. Some antidepressants (e.g., SSRIs) may reduce the efficacy of breast cancer treatments and cause side effects such as drowsiness, weight gain, and sexual dysfunction, potentially interfering with standard therapy [10].

Increasingly, research indicates that natural herbal medicines, particularly those in traditional Chinese medicine (TCM), may offer more comprehensive, multi-target treatments with fewer side effects [11]. Among these, Jiawei Suanzaoren (JWSZR), a traditional herbal formula, has gained attention for its neuroprotective effects. Composed mainly of jujube seed, licorice root, and Chuanxiong [12, 13], JWSZR is known for its sedative, calming, and sleep-improving properties. It is one of the most commonly used traditional Chinese formulas for insomnia, anxiety, and night sweats [14, 15]. Preliminary studies have demonstrated its neuroprotective effects, particularly in modulating neuroinflammation and inhibiting apoptosis [16], providing a strong theoretical basis for its application in treating depression. Notably, Ziziphus jujuba seed, a key component of JWSZR, has been shown in multiple studies to possess significant sedative, anxiolytic, and sleep-improving effects [17, 18]. However, the specific mechanisms of JWSZR in depression, particularly its multi-target regulatory pathways, have yet to be systematically explored. Therefore, further elucidation of its mechanisms, especially its roles in immune regulation and neuroprotection, is significant for advancing depression treatment.

While many studies on TCM formulas have shown multi-target neuroprotective effects on the nervous system, the specific molecular mechanisms of JWSZR remain unclear, particularly its multi-target regulatory mechanisms in treating depression. Current research indicates that TCM formulas exert neuroprotective effects primarily by modulating inflammatory responses, inhibiting oxidative stress, and regulating cell survival signaling pathways through various mechanisms [19–21]. Additionally, applying advanced techniques like network pharmacology and molecular docking further elucidates the action networks of TCM formulas in neuroprotection [22], allowing a systematic clarification of their potential mechanisms. Constructing protein–protein interaction (PPI) networks and identifying core targets [23, 24] help pinpoint JWSZR's role in depression-related pathways, while molecular docking analysis further validates the binding patterns between JWSZR's main components and specific targets. This research design not only aids in understanding the multi-target synergistic effects of TCM formulas but also provides new directions for subsequent drug development. However, current studies largely focus on individual components or specific pharmacological effects of JWSZR, lacking a comprehensive analysis of its overall mechanism. Therefore, this study aims to systematically unveil the multi-target neuroprotective mechanisms of JWSZR in depression through a multi-tiered approach involving network pharmacology, molecular docking, and in vitro experiments, addressing gaps in existing research.

This study systematically investigates the multi-target neuroprotective mechanisms of JWSZR in depression by integrating network pharmacology, molecular docking, and in vitro validation, offering a comprehensive approach beyond traditional single-target studies. First, TCMSP and HERB databases identify six key active components of JWSZR, which are mapped to depression-related targets from GeneCard, DisGeNET, and OMIM databases. A PPI network is constructed to pinpoint potential key targets. Next, AutoDock Vina is employed for molecular docking, verifying the binding interactions between JWSZR components and depression-related proteins. Finally, an in vitro corticosterone (CORT)-induced PC12 cell model is used to assess JWSZR's neuroprotective effects, evaluating changes in cell morphology, survival, and apoptosis.

Unlike previous studies focusing on single compounds, this study employs a multi-tiered, integrative strategy to systematically elucidate JWSZR's synergistic mechanisms in depression treatment. The findings provide a scientific basis for the potential use of JWSZR as a novel adjunctive therapy, advancing the application of traditional medicine in neuropsychiatric disorders and supporting future clinical research.

Materials and methods

Screening of JWSZR active components and target prediction

JWSZR's chemical components were retrieved from the TCMSP (https://old.tcmsp-e.com/tcmsp.php) and HERB (http://herb.ac.cn) databases, with manual literature review confirming active compounds and corresponding protein targets. Active components were filtered based on oral bioavailability (OB $\geq$20%) and drug-likeness (DL $\geq$0.18), criteria derived from extensive TCM compound analyses and relevant literature to ensure good absorption, bioactivity, and drug development potential. Selected compounds were mapped to the STRING

database (https://string-db.org) to identify corresponding protein targets and ensure reliability for compound-target and PPI network construction (Table 1) [25–28].

Screening of depression-related targets and Venn diagram analysis

Potential depression-related targets were identified by searching the keywords"psychological depression"and"depression"in the DisGeNET (https://www.disgenet.org), GeneCards (https://www.genecards.org), and OMIM (https://omim.org) databases. Duplicate removal was performed to integrate targets from different databases, ensuring each target appeared only once. The de-duplication process was based on standardized gene names or identifiers rather than relying on database-specific identifiers. Venny 2.1.0 was then used to generate a Venn diagram, identifying the intersection between JWSZR component targets and depression-related targets, thereby screening potential therapeutic targets of JWSZR in depression treatment.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

GO and KEGG enrichment analyses were performed on the targets within the PPI network using the Metascape platform. The parameters were set as follows: p-value <0.05, minimum gene count of 3, and enrichment factor >1.5, with multiple testing corrections using the Benjamini–Hochberg method. Kappa scores were used as a similarity measure for hierarchical clustering of enriched terms, grouping terms with a similarity >0.3 into the same cluster. Visualization was conducted in Cytoscape, where each node represents an enriched term, and edges indicate the similarity between terms.

Construction of the JWSZR active component-target network and PPI network

The selected common targets were uploaded to the STRING v11.0 platform with the species restricted to **Homo sapiens* and an interaction score threshold set at >0.9 to ensure high-confidence interactions within the network. The resulting PPI network data were then imported into Cytoscape 3.9.1, where topological analysis was conducted based on degree values to identify key targets and active components within the network. Additionally, the constructed active component-target network provides visual support for identifying multi-target mechanisms of action.

Molecular docking analysis

Molecular docking analysis was performed using AutoDock Vina to investigate the binding interactions between key JWSZR components and target proteins. The three-dimensional (3D) structures of target proteins were retrieved from the PDB database (https://www.rcsb.org/), with PDB IDs 1U3Q (ESR2) and 5H22 (HSP90 AA1). After obtaining the target protein structures, PyMOL was used to remove ligands and water molecules, followed by preprocessing in AutoDock Tools, where polar hydrogen atoms and Gasteiger charges were added. The 3D structures of active compounds were obtained from the PubChem database and optimized via energy minimization using Chem3D. Docking simulations were conducted at the active site of the target proteins using a grid box with nine independent docking runs. Results were ranked based on binding energy, and the optimal binding conformations were evaluated through hydrogen bonding and hydrophobic interactions. The final docking results were visualized using PyMOL.

PC12 cell culture and drug treatment

PC12 cells were purchased from ATCC (American Type Culture Collection, USA) and cultured according to the supplier's recommended protocol in high-glucose Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin–streptomycin solution (Gibco, USA), maintained at 37 °C in a 5% CO_2 incubator. When cell confluency reached 80–90%, cells were digested with

Table 1 Active components of JWSZR decoction and corresponding targets

Pharmaceutical name	Chinese name	Latin botanical name	TSCMP		HERB	
			Compounds	*Targets*	*Compounds*	*Targets*
Semen Ziziphi Spinosae	酸枣仁 (*Suanzaoren*)	*Ziziphus jujuba Mill. var. spinosa*	12	42	–	–
Sclerotium Poriae Cocos	茯苓 (*Fuling*)	*Poria cocos (Schw.) Wolf*	6	27	–	–
Radix Ligustici Chuanxiong	川芎 (*Chuanxiong*)	*Ligusticum chuanxiong Hort*	20	109	–	–
Rhizoma Anemarrhenae	知母 (*Zhimu*)	*Anemarrhena asphodeloides Bge*	17	107	–	–
Radix Glycyrrhizae	甘草 (*Gancao*)	*Glycyrrhiza uralensis Fisch*	92	227	–	–
Amber	琥珀 (*Hupo*)	*Succinum*	–	–	4	24

0.25% trypsin–EDTA (Gibco, USA) for subculturing or experimental treatments.

Experimental groups included the control (basic medium), corticosterone (CORT) group (200 μM CORT treatment), JWSZR + CORT group (0.1 mg/mL JWSZR treatment), and ESZL + CORT group (100 μM eszopiclone treatment). Drug treatments were administered for 24, 48, 72, and 96 h to assess cell viability and apoptosis at different times.

JWSZR consisted of one packet of JWSZR (Batch No: 2107020, Xinluyao) and half a packet of amber (Batch No: 2100606, Xinluyao). The mixture was homogenized, and 2 g was measured, mixed with 1 mL distilled water, and shaken thoroughly. It was then centrifuged at 2000 rpm for 30 min, and the supernatant was filtered and stored at 4 °C as a 2 g/mL stock solution with a working concentration of 0.65 mg/mL. Eszopiclone (ESZL) was obtained from Changle Pharmaceutical Co., Ltd. (Xinxiang, China) with a molecular weight of 294.74; each tablet contained 1 mg. A 100 mM stock solution was prepared by dissolving the tablet in 34 μL DMSO and was used at a final concentration of 100 μM in cell experiments.

Cell viability assay

Cell viability was assessed using a Cell Counting Kit-8 (CCK-8) kit (Beyotime, China). PC12 cells were seeded at a density of 1×10^5 cells/mL in a 96-well plate, with 100 μL of cell suspension added to each well. After attachment, cells were treated according to the experimental design. Following 24 h of treatment, 10 μL of CCK-8 solution was added to each well, and the plate was incubated at 37 °C in the dark for 2 h. Absorbance was measured at 450 nm using a microplate reader to evaluate cell viability.

Apoptosis and cell cycle detection

Apoptosis and cell cycle distribution were analyzed using flow cytometry. Treated PC12 cells were collected and washed with pre-cooled PBS (Thermo Fisher, USA). Cells were then fixed in 70% ethanol (Sigma-Aldrich, USA) and stored at 4 °C for 2 h before treatment with PI staining buffer containing 50 μg/mL propidium iodide (PI, Sigma-Aldrich, USA) and 100 μg/mL RNase A (Thermo Fisher, USA). Samples were analyzed on a BD FACSCalibur flow cytometer (BD Biosciences, USA), and red fluorescence signals were recorded in the FL-2 channel. Data were analyzed using FlowJo software to determine apoptosis rates and cell cycle distribution.

Statistical analysis

Experimental data were analyzed using GraphPad Prism 8.0. All data are presented as mean ± standard deviation (Mean ± SD). Differences between groups were evaluated using one-way analysis of variance (ANOVA), with Tukey's multiple comparison test applied to determine significance levels. A significance threshold was set at $p < 0.05$. Normality and homogeneity of variance tests were performed before analysis to ensure the accuracy and reliability of the statistical assessments.

Results

Multi-component, multi-target mechanism analysis based on network pharmacology reveals potential therapeutic value of JWSZR in depression

This study used network pharmacology to systematically analyze the multi-target interactions between JWSZR active components and depression-associated targets, integrating multiple public databases and literature sources to elucidate potential mechanisms (Fig. 1A).

Initially, six primary active components and 290 target proteins of JWSZR were identified from TCMSP and HERB databases, supplemented by a manual literature review. Depression-related targets were retrieved from GeneCards, DisGeNET, and OMIM, yielding 9,485 disease-related targets after integration and deduplication. Venn diagram analysis identified 251 overlapping targets between JWSZR and depression, suggesting potential relevance in depression-related disorders (Fig. 1B).

GO and KEGG enrichment analyses were performed to explore the functional implications of these targets. GO analysis categorized gene functions into Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) (Fig. 1C). BP terms were associated with metabolic and stress response processes, CC terms with cell membrane and extracellular regions, and MF terms with enzyme activity and receptor binding. KEGG pathway analysis (Fig. 1D) revealed enrichment in pathways related to thyroid cancer, endocrine resistance, prolactin, and estrogen signaling, as well as longevity regulation and neural-immune signaling. These findings indicate the broad involvement of JWSZR-related targets in metabolic regulation, cellular signaling, and disease-associated pathways.

Analysis of JWSZR PPI network and key functional modules

A PPI network was constructed using 251 depression-related targets identified from GeneCard, DisGeNET, and OMIM. The network, generated via STRING (confidence score ≥ 0.9), contained 256 nodes and 797 edges (Fig. 2A). Core proteins, including TP53 and JUN, exhibited high connectivity, suggesting their central regulatory roles. MCODE analysis identified highly clustered functional modules, with the Rank 1 module (Fig. 2B) scoring 7.655, indicating strong functional relevance. TP53 and JUN were prominent within this module, while ER and HSP90 AA1

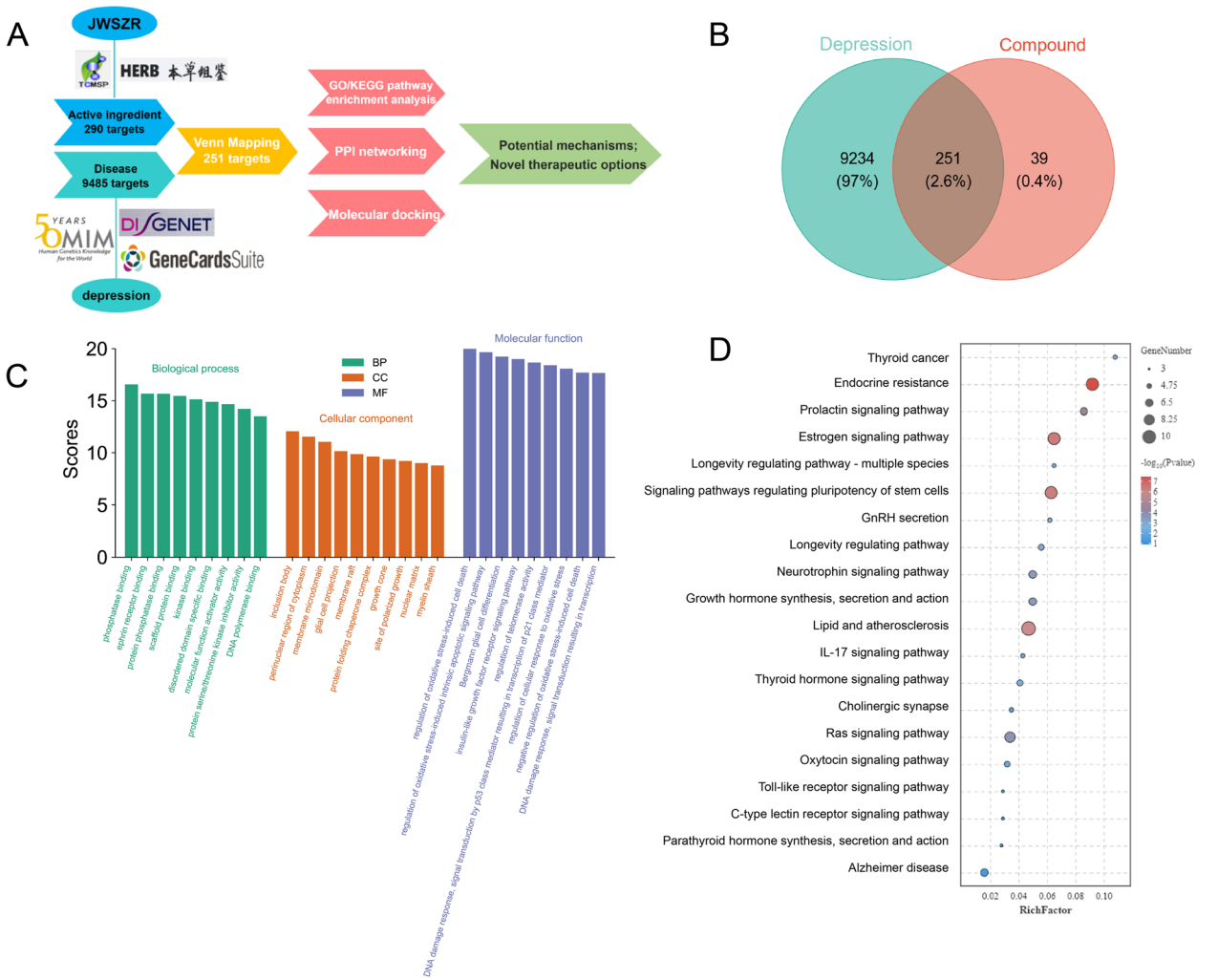


Fig. 1 Screening of Active Components and Disease Targets of JWSZR with GO and KEGG Enrichment Analysis. Note: (**A**) Flowchart of network pharmacology analysis for screening active components of JWSZR and identifying depression-related disease targets. **B** Venn diagram illustrating the overlap between targets of JWSZR active compounds and depression-related targets. **C** GO enrichment analysis shows functional classifications of JWSZR-related targets in BP, CC, and MF. Statistical significance was assessed using a two-tailed *p*-value, with $p < 0.05$ considered significant. **D** KEGG pathway enrichment analysis reveals the major biological pathways involving JWSZR targets. Enrichment was evaluated by Rich factor; dot size indicates the number of target genes, and color represents the significance level as $-\log_{10}(p\text{-value})$

were identified as potential targets in immune regulation and inflammatory pathways.

GO and KEGG enrichment analyses (Table 2, Fig. 2C) revealed that target proteins were predominantly associated with apoptosis regulation, immune response, and neuroprotection. KEGG pathway analysis highlighted the involvement of MAPK, JAK-STAT, and BDNF pathways, which are linked to neuroinflammation, immune modulation, and neuroplasticity.

Molecular docking study of JWSZR main components with key target proteins reveals high-affinity binding patterns in depression

Molecular docking analysis was conducted using AutoDock Vina to investigate the interactions between JWSZR active components and key depression-related targets. A component-target network was constructed (Fig. 3A), identifying high-degree active components such as licorice root, Poria, and Chuanxiong (Table 3). Hederagenin, a major component, exhibited interactions with multiple target proteins, suggesting its involvement in multi-target regulation.

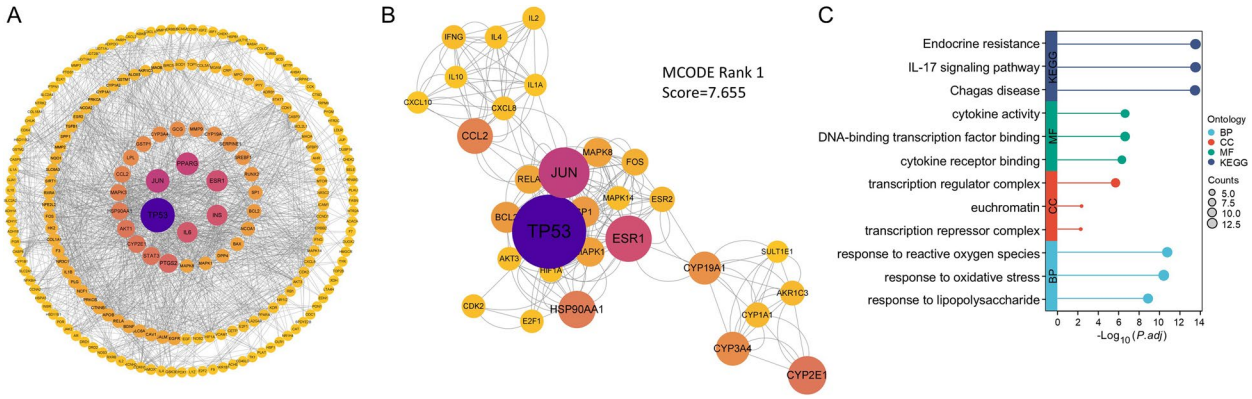


Fig. 2 PPI Network and Functional Module Analysis of JWSZR. Note: (**A**) Construction of the PPI network. Node size and color intensity indicate the importance of target proteins within the network, with purple nodes representing central targets and yellow nodes representing secondary targets. **B** Display of the MCODE Rank 1 module. The top-scoring functional module (Rank 1, score = 7.655) identified by the MCODE algorithm highlights the positions and relative connectivity of core targets such as TP53, JUN, and ESR1 within the module. **C** GO and KEGG pathway enrichment analysis results for targets within the MCODE Rank 1 module. Dot size indicates the number of genes, while color represents the significance level as -$\log_{10}$(*p*-value)

Docking simulations were performed for three key active components and two major target proteins (ESR2 and HSP90 AA1). All components displayed binding energies below −5.0 kJ/mol, indicating strong affinity (Table 4). Hederagenin-ESR2 interaction showed the lowest binding energy (−15 kJ/mol), with docking analysis revealing hydrogen bonds and hydrophobic interactions at GLU-206, ASN-234, and ARG-301 residues (Fig. 3B-E). Other active components also exhibited high binding affinity with ESR2 and HSP90 AA1, supporting their potential involvement in protein interactions and pathway modulation.

Validation of the neuroprotective effect of JWSZR in a CORT-Induced PC12 cell injury model

In vitro experiments using a CORT-induced PC12 cell model were conducted to assess JWSZR's effects on cell viability and apoptosis. Morphological observations showed that CORT-treated cells exhibited shrinkage and detachment, indicating cellular damage, while JWSZR and ESZL treatments improved cell morphology (Fig. 4A). Cell viability analysis using the CCK-8 assay demonstrated that JWSZR and ESZL significantly increased viability compared to the CORT group, with JWSZR showing stronger effects at 72 and 96 h (Fig. 4B). Flow cytometry analysis revealed that CORT treatment significantly increased apoptosis, whereas JWSZR and ESZL reduced apoptosis rates. JWSZR exhibited a greater apoptosis-inhibiting effect than ESZL (Fig. 4C-D).

Discussion

In the field of depression treatment, current medications primarily target single pathways to alleviate symptoms, such as SSRIs [29]. Although effective, these treatments are often associated with significant side effects and high relapse rates [6, 7]. Although ketamine is used clinically as a rapid-acting antidepressant, its abuse potential and dissociative side effects limit its suitability for routine treatment [30]. Advancements in neuroscience and pharmacology have revealed that depression involves multiple molecular pathways, including immune response, neuroinflammation, neurotransmitter imbalance, and apoptosis [31–33]. Key signaling pathways such as PI3 K-Akt, calcium signaling, MAPK, estrogen signaling, and apoptosis significantly contribute to type II diabetes progression [34]. Given this complexity, single-target therapies are insufficient to fully address the pathophysiology of depression. Multi-component, multi-target TCM formulas are increasingly gaining attention for their synergistic effects, showing promise in neuroprotection and immune balance modulation. This study systematically explores the multi-target neuroprotective mechanisms of JWSZR in depression treatment through network pharmacology, molecular docking, and in vitro experiments, uncovering its roles in regulating key target proteins (such as STAT1, MAPK1, and CASP3), modulating immune responses, and suppressing neuroinflammation.

This study identifies the multi-target neuroprotective effects of JWSZR in depression and highlights key pathways involved in its mechanism. Among these, endocrine resistance and the IL-17 signaling pathway play critical roles in depression pathophysiology. Endocrine resistance is linked to hormonal imbalance, which

Table 2 Highly regulated core genes in protein–protein interaction (PPI) networks

name	Degree	Betweenness	Closeness	MCODE::Score
TP53	104	8611.812234	0.06547619	5.8
JUN	68	4742.065385	0.065320665	6.095238095
HSP90 AA1	62	2507.254498	0.064327485	5.785714286
ESR1	58	3992.634294	0.064992614	6.32967033
MAPK1	48	1171.832036	0.064591897	6.241758242
BCL2	42	1458.578476	0.063953488	6.222222222
RELA	38	1046.561748	0.064383963	7
FOS	38	756.9835973	0.064308682	5.939393939
CYP3 A4	36	1933.241874	0.061094141	6.066666667
CYP2E1	36	2695.097672	0.061884669	6
MAPK8	34	1151.791328	0.064308682	7
AKT3	34	246.0359676	0.063712714	5.785714286
SP1	32	1467.509505	0.064158647	6.564102564
HIF1 A	32	185.6144626	0.064102564	6.109090909
CYP1 A1	30	484.509968	0.060857538	6.066666667
IFNG	30	302.1025177	0.062482249	6.533333333
MAPK14	30	296.8398564	0.063712714	6.533333333
CXCL8	28	276.8652943	0.063218391	6.533333333
CCL2	28	2245.875601	0.063037249	6.805555556
ESR2	24	522.7170549	0.063528732	7
E2 F1	24	212.7148455	0.062429058	6.416666667
CDK2	24	255.600901	0.062076749	6.416666667
IL10	22	52.272507	0.062446778	6.533333333
IL1 A	22	44.0540018	0.062606716	6.533333333
AKR1 C3	22	435.0013553	0.059863946	6.066666667
IL4	20	154.0198788	0.062252405	6.805555556
IL2	18	133.1529404	0.061641917	7
CYP19 A1	18	1780.63208	0.062270025	6
CXCL10	18	7.940740741	0.061401061	7
SULT1E1	14	0.5	0.05952381	6

may exacerbate depressive symptoms, while the IL-17 pathway is associated with immune and inflammatory responses, both of which are implicated in depression development [35–37]. Dysregulated immune responses are recognized as potential contributors to depression, emphasizing the therapeutic relevance of these pathways. Findings suggest that JWSZR modulates these pathways, potentially alleviating immune dysregulation and neuroinflammation associated with depression.

The results of this study align with previous research on the neuroprotective effects of TCM formulas, consistently showing that JWSZR modulates immune responses and alleviates inflammatory damage [38–41]. Unlike single-target or single-component studies, this research further reveals JWSZR's coordinated regulation of multiple key targets. For instance, Hederagenin, a key active component of JWSZR, regulates the apoptotic pathway through specific binding to ESR2. Hederagenin and estradiol exhibit ESR2-binding affinity, yet their molecular structures differ significantly. Estradiol, a classical estrogen, contains a benzene ring and phenolic hydroxyl group, which are essential for ESR2 interaction [42–44]. In contrast, Hederagenin, a triterpenoid, features a pentacyclic terpenoid skeleton with multiple hydroxyl groups. Although it lacks a benzene ring, its hydrogen bond donors and acceptors may facilitate interactions with specific amino acid residues at the ESR2 binding site.

During the research process, we found that the binding energy of Hederagenin in JWSZR with ESR2 was significantly lower than that of other components. This unexpected finding suggests that Hederagenin may hold a unique position in the multi-target mechanism of JWSZR. Such specific binding of Hederagenin may help stabilize the ER signaling pathway, thereby enhancing the neuroprotective effects of JWSZR. Studies have shown that estrogen receptors regulate serotonin (5-HT) synthesis and reuptake, enhance glutamate release, and modulate dopamine (DA) synthesis and release. Dysfunction in the 5-HT, glutamate, and DA systems, mediated by the PI3 K and MAPK signaling pathways, is closely associated with depression [45]. Compared with other studies, we believe that Hederagenin's binding pattern in the ER pathway could be the source of its distinct biological effects. However, this finding also highlights the need for future research to further explore Hederagenin's independent contribution to neuroprotection, elucidating its key role within JWSZR's multi-target mechanism.

The findings of this study have potential clinical implications. Depression is a common and complex disorder, and current pharmacological treatments often have side effects and high relapse rates, highlighting the need for safer alternatives. This study demonstrates that JWSZR exerts neuroprotective effects against corticosterone-induced injury through multi-target regulation and immune modulation. While in vitro and computational results are promising, further in vivo studies and clinical trials are needed to confirm its therapeutic potential and safety. Additionally, investigating its combination with conventional antidepressants could provide insights into its role as an adjunct therapy, particularly for mild to moderate depression, potentially reducing reliance on traditional antidepressants.

This study has certain limitations. First, it primarily relies on network pharmacology and molecular docking, which, while providing a theoretical framework for multi-target mechanisms, require further in vivo validation. Additionally, the small sample size in in vitro experiments may introduce selection bias, limiting the generalizability of the findings. Computational errors in

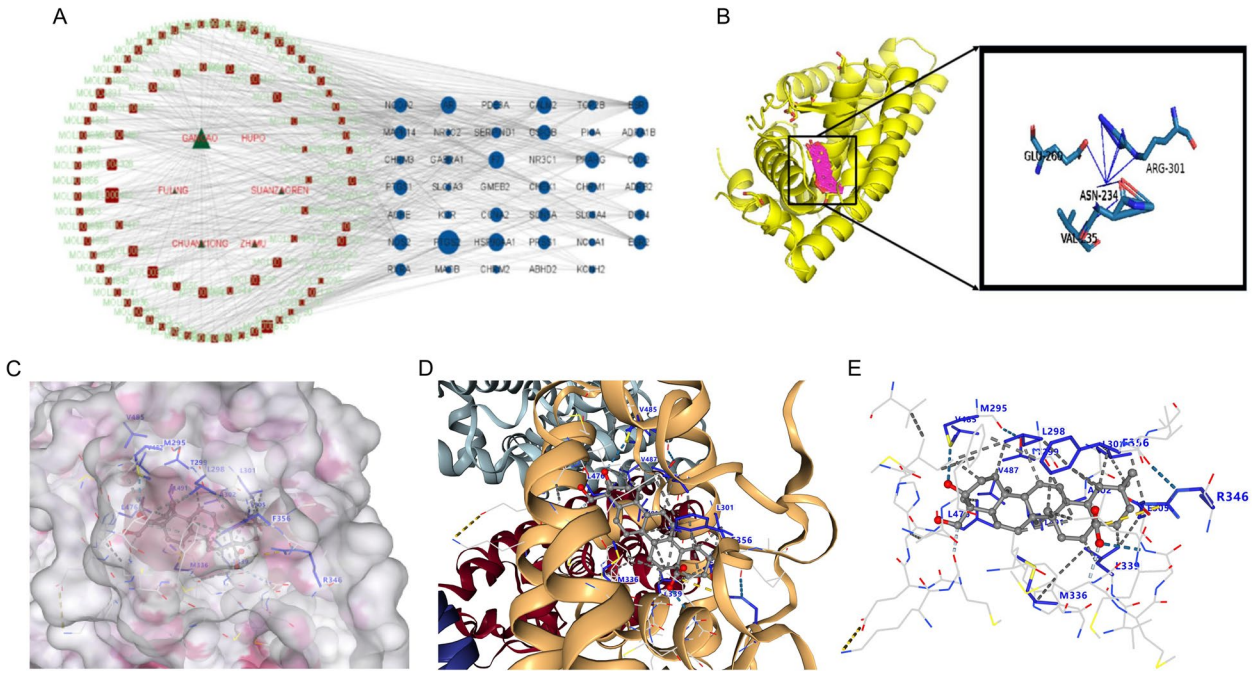


Fig. 3 Molecular Docking Analysis of Major Active Components and Key Target Proteins in JWSZR. Note: (**A**) Component-target network illustrating the top three active components in JWSZR by degree value (licorice root, poria, Chuanxiong) and their interactions with key target proteins. Node size is proportional to degree value, and edge thickness represents the strength of interactions between targets and components. **B**-**E** Molecular docking model of Hederagenin with ESR2. Molecular docking was conducted using AutoDock Vina, with binding modes selected based on the lowest binding energy, highlighting hydrogen bonds and hydrophobic interactions. Hydrogen bonds are formed at residues GLU-206, ASN-234, and ARG-301 in the model

Table 3 Effective active components of JWSZR decoction

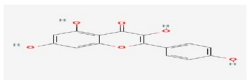
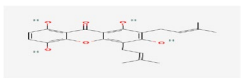
No	Active Component	Degree Value	Source Herb	Structure
1	MOL000422 kaempferol	17	Zhimu (Anemarrhena asphodeloides)	
2	MOL000467 Gartanin	15	Gancao (Glycyrrhiza glabra)	
3	MOL000296 hederagenin	11	Fuling (Poria)	

Table 4 Minimum binding energies between key active components and targets

Active Component			Binding Energy (kcal/mol)		Total Score
Name	Chemical Formula	Molecular weight	HSP90 AA1	ESR2	
Kaempferol	$C_{15}H_{10}O_6$	286.24	−9.4	−9.3	−18.7
Gartanin	$C_{23}H_{24}O_6$	396.4	−9.6	−9.6	−19.2
Hederagenin	$C_{30}H_{48}O_4$	472.7	−13.8	−15	−28.8
Total Score			−32.8	−33.9	

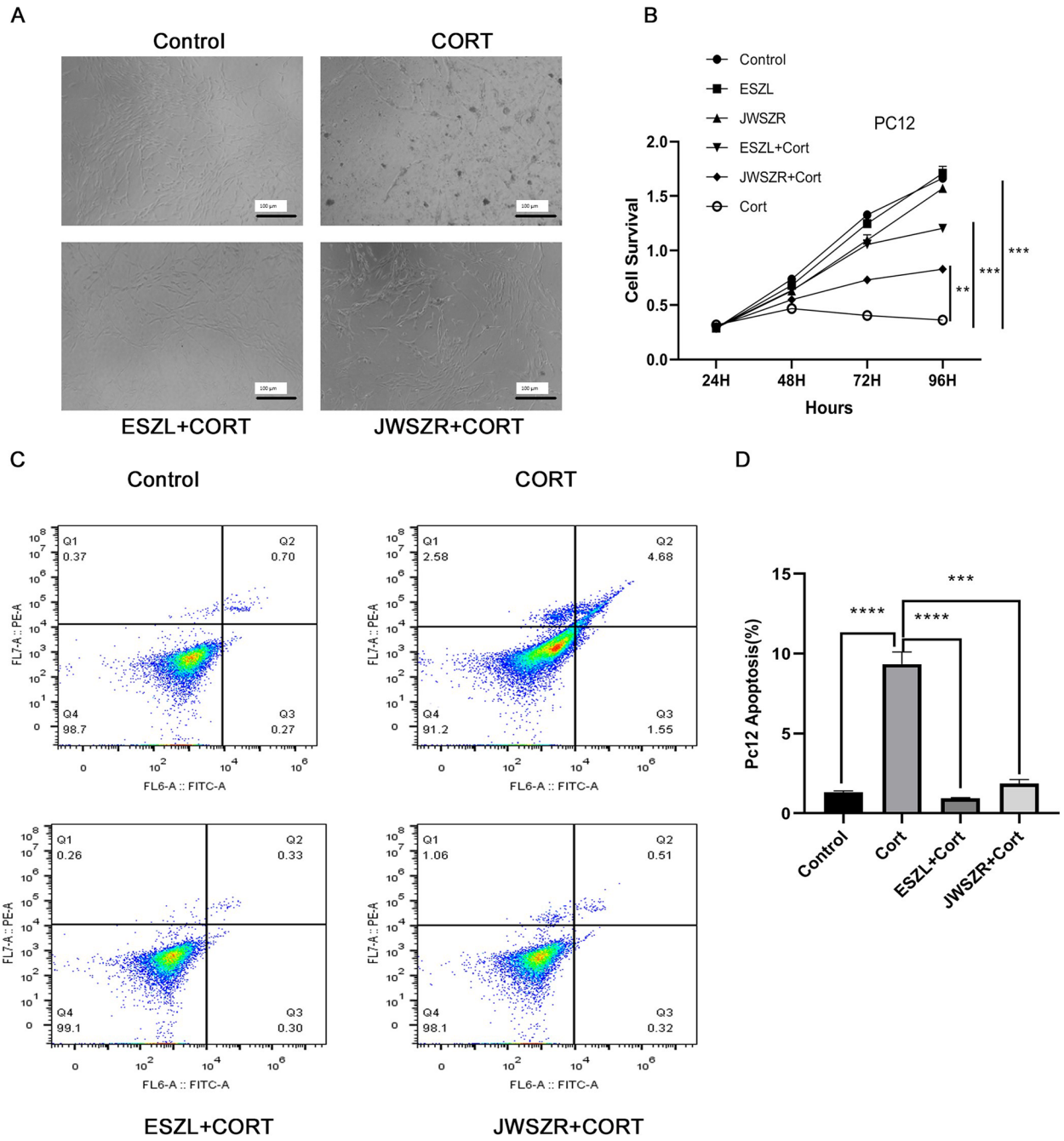


Fig. 4 Validation of the Neuroprotective Effect of JWSZR on CORT-Induced PC12 Cell Injury. Note: (**A**) Morphological changes in PC12 cells observed by optical microscopy across different groups. PC12 cells were treated with CORT for 24 h to induce cell injury. Experimental groups included Control, CORT-treated, JWSZR-treated, and ESZL-treated groups. **B** CCK-8 assay evaluates the survival rate of PC12 cells at various time points (24, 72, and 96 h). Data are presented as Mean ± SD ($n = 3$), with statistical significance assessed by a two-tailed *t*-test, $p < 0.05$ considered significant. **C** Flow cytometry analysis of apoptosis rates in PC12 cells across groups, measuring early and late apoptosis rates. Data are presented as Mean ± SD ($n = 3$). **D** Quantitative results of apoptosis analysis. Differences between groups were evaluated by a two-tailed *t*-test, with significance levels indicated as **** for $p < 0.0001$, *** for $p < 0.001$, and ** for $p < 0.01$

molecular docking could also affect the accuracy of target-binding predictions. Furthermore, although JWSZR shows potential as an adjunct therapy, its integration with existing antidepressant treatments and the individual variability in treatment response were not explored. Future research should expand sample sizes, incorporate

in vivo studies, and further investigate JWSZR's neuroprotective effects.

Further studies should investigate JWSZR's molecular mechanisms in depression, particularly its role in cellular signaling pathways. Hederagenin, identified as a key active component, may interact with ESR2, potentially influencing serotonin signaling and neuroplasticity [46, 47], warranting further mechanistic studies. Additionally, multi-omics approaches, such as metabolomics and proteomics, could help elucidate the synergistic effects of JWSZR's components. Clinical research should focus on large-scale trials to assess safety, efficacy, and potential drug interactions, particularly concerning organ toxicity and compatibility with conventional antidepressants. Establishing a multidisciplinary research platform integrating pharmacology, psychiatry, and clinical medicine will be essential for optimizing JWSZR's clinical application and facilitating its therapeutic translation.

In conclusion, this study systematically reveals the multi-target neuroprotective effects of JWSZR in depression, expanding the potential of TCM formulas in treating neuropsychiatric disorders. By regulating key target proteins, JWSZR achieves immune modulation, anti-inflammatory, and neuroprotective effects, offering a new multi-target adjunctive therapy option for depression treatment. This research enhances theoretical understanding of the mechanisms of TCM formulas and holds significant clinical value. However, the findings still require further validation through in vivo and clinical trials, and closer links between mechanistic studies and large-scale clinical applications are needed to advance the practical use of JWSZR in depression therapy.

Conclusion

This study systematically validated the multi-target neuroprotective effects of JWSZR in depression using network pharmacology, molecular docking, and in vitro experiments. PPI network analysis identified key targets (ESR2, STAT1, CASP3) interacting with JWSZR components, suggesting modulation of immune signaling and neurotrophic pathways to maintain cell survival and reduce neurotoxicity. Molecular docking confirmed high-affinity binding, particularly Hederagenin-ESR2 interactions, stabilizing signaling through hydrogen bonding and hydrophobic interactions. In vitro experiments demonstrated that JWSZR enhanced cell viability and inhibited apoptosis in a CORT-induced PC12 cell injury model, exhibiting superior neuroprotection compared to ESZL (Graphic abstract).

Despite these findings, in vivo and clinical validation is needed to confirm JWSZR's efficacy and safety. Future research should focus on clinical trials, biomarker analysis, and long-term evaluations to explore its therapeutic potential and personalized treatment strategies.

Abbreviations

ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BP	Biological Process
CC	Cellular Component
CCK-8	Cell Counting Kit-8
CORT	Corticosterone
DL	Drug-Likeness
DMEM	Dulbecco's Modified Eagle Medium
ESZL	Eszopiclone
FBS	Fetal Bovine Serum
GO	Gene Ontology
JWSZR	Jiawei Suanzaoren
KEGG	Kyoto Encyclopedia of Genes and Genomes
MF	Molecular Function
Mean ± SD	Mean ± Standard Deviation
OB	Oral Bioavailability
PI	Propidium Iodide
PPI	Protein–Protein Interaction
SSRIs	Selective Serotonin Reuptake Inhibitors
TCM	Traditional Chinese Medicine

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None.

Authors' contributions

Ruiting Ma and Wenjing Zhang contributed equally to this work and are regarded as co-first authors. Ruiting Ma was responsible for data collection, network pharmacology analysis, and manuscript drafting. Wenjing Zhang performed the molecular docking studies and contributed to data interpretation. Lixia Chen supervised the experimental design, conducted the in vitro validation, and critically revised the manuscript. Lijun Tong conceptualized the study, provided project administration and funding acquisition, and oversaw the overall research execution. All authors read and approved the final manuscript.

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Data availability

All the Raw data is available at Figshare: https://doi.org/10.6084/m9.figshare.28768487.

Declarations

Ethics approval and consent to participate

No ethical approval is required for this study.

Competing interests

The authors declare no competing interests.

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