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ORIGINAL RESEARCH

Danggui Buxue Decoction Alleviates Acute Hypoxia-Induced Sleep Disorders: Insights from in vivo and in silico Analyses

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Background: Acute hypoxia-induced sleep disorders (AHISD) frequently occur in individuals exposed to high altitudes, yet current treatment options are limited. Danggui Buxue decoction (DBD) originated from the ancient medical book "NeiWaiShangBianHuo Lun" for more than 700 years, and is widely used to treat anemic diseases in clinical, with the potential to treat AHISD.

Methods: We employed a combination of behavioral assays, transcriptomics, neurotransmitter profiling, and in-silico approaches to evaluate the effects and mechanisms of DBD.

Results: DBD supplementation significantly alleviated sleep disorders in AHISD fruit flies, restoring the sleep time and the sleep structure. Mechanistically, DBD alleviated AHISD mainly by restoring neurotransmitter balance such as Glutamine (Gln) and Glutamic acid (Glu), modulating serotonin receptors (5-HT1A/1B), and suppressing neuroinflammatory Toll and Imd pathways. In addition, resveratrol, magnolol, and caffeic acid as the main active ingredients of DBD played an important role in alleviating AHISD.

Conclusion: These findings suggest DBD as a promising candidate for mitigating AHISD through neuroprotective and anti-inflammatory mechanisms.

Keywords: Danggui Buxue decoction, acute hypoxia-induced sleep disorder, neuroprotective mechanism, active ingredients, *Drosophila melanogaster*

Introduction

As more individuals are traveling to plateau regions for work or leisure, acute hypoxia-induced sleep disorders (AHISD) often occur due to the decreased oxygen concentration.[1–3] Although drug intervention is the primary form of treatment for AHISD, there are still issues such as limited treatment options and side effects.[4] Therefore, it is crucial to find effective intervention measures for AHISD.

The pathogenesis of AHISD is a complex process, and current studies have mainly focused on neurotransmitter disorders and neuroinflammation.[5,6] The dynamic balance between sleep and wakefulness depends on the coordinated action of multiple neurotransmitters.[7] Hypoxia alters the expressions of TH, ChAT and GAD enzyme, which is associated with sleep neural homeostasis in various brain regions, leading to the elevated dopamine levels and reduced serotonin (5-HT) levels in the frontal cortex, pons, and medulla, ultimately decreasing REM sleep duration and increasing sleep latency.[5] Researchers suggest that high altitude hypoxia induces an increase cytokines of brain such as IL-1, IL-6, and TNF-α by stimulating EPO production, ultimately altering sleep quality and structure.[8] Thus, the modulation of signaling pathways by targeted drugs is considered a promising strategy for the treatment of AHISD.

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Danggui Buxue decoction (DBD), a famous formula composed of *Angelicae Sinensis Radix* (Danggui, DG) and *Astragali Radix* (Huangqi, HQ) in a ratio of 1:5, is originated from the "NeiWaiShangBianHuo Lun" written by Li Dongyuan in the 13th century.[9] It has been widely used to treat anemia for more than 700 years. Modern pharmacological research has shown that DBD has the ability to improve brain diseases. For instance, DBD could improve depressive symptoms in GK rats by activating the CREB/BDNF/TrkB signaling pathway;[10] DBD combined with endothelial progenitor cells could promote the reconstruction of neural function to treat focal cerebral ischemia;[11] DBD could improve the sleep scale scores of Parkinson's patients and enhance their sleep quality.[12] In addition, *Astragali Radix* and *Angelicae Sinensis Radix* could regulate 5-HT levels to alleviate the disease.[13,14] DBD consists of a variety of active ingredients, among which quercetin, ferulic acid and kaempferol could regulate GABA and 5-hydroxytryptaminergic system to improve sleep quality.[15–18] However, there are few studies regarding the protective effect of DBD on AHISD. Thus, it is essential to explore the role, mechanism and active ingredients of DBD in the treatment of AHISD.

Currently, various animal models have been used to dissect the pathogenesis of diseases and screen drugs, among which the fruit fly (*Drosophila melanogaster*) is considered a rapid and systematic in vivo model for studying the sleep disorders.[19,20] It particularly shares evolutionary conserved mechanisms regulating sleep homeostasis with human,[21–23] such as the involvement of 5-HT and dopamine receptors in sleep-awake state[24,25] and a crosstalk relationship between inflammation and sleep.[26] Multiple studies had used fruit fly model to confirm that moderate hypoxia is beneficial for increasing sleep time, reducing sleep frequency, and improving sleep quality.[27,28] Therefore, flies can be used to screen new therapeutic agents and investigate molecular mechanisms of AHISD. With the development of bioinformatics, the combined analysis of transcriptomics and clinical datasets could reveal the conserved pathogenic mechanisms of diseases across different species, for instance, chemotherapeutic intestinal injury in *Drosophila* has conserved pathogenic mechanisms such as Toll-like receptor signalling pathway, autophagy, and apoptosis.[29] In addition, the use of traditional Chinese medicine whole target mass spectrometry analysis, pharmacological feature prediction, network pharmacology, and molecular docking could effectively screen active ingredients and analyze potential mechanisms.[30,31] The integrated research of multi-technology could provide a comprehensive understanding of the therapeutic mechanism and material basis of DBD.

Accordingly, this study firstly evaluated the therapeutic effects of DBD on AHISD. The mechanism and functional compounds of DBD were then clarified combined with in vivo experiments and in-silico approaches, offering a novel intervention method for the clinical treatment of AHISD.

Materials and Methods

Experimental Reagents

Astragali Radix (Gansu Province, China, batch number 2307007) and *Angelicae Sinensis Radix* (Gansu Province, China, batch number 20230801) were purchased from and identified by the Affiliated Hospital of Gansu University of Chinese Medicine, which were in line with the standards listed in the National Pharmacopoeia of China. The separation, concentration, lyophilization and storage of DBD were performed according to previous research.[32] In brief, *Angelicae Sinensis Radix* slices and *Astragali Radix* slices were mixed in a ratio of 1:5 (w/w). They were immersed in ultra-ultra-pure water (1:8, w/v) for 1 h, boiled for 1 h and repeated twice. The obtained aqueous extracts were evaporated, concentrated and placed in a lyophiliser to collect the lyophilised powders at −80°C. Based on the previous studies and pre-experiments, the concentration of DBD was used as 2.5, 10 and 40 mg/mL, respectively.[32,33] Small molecules of DBD were purchased from Shanghai Yuanye Bio-Technology Co (Shanghai, China) (Table 1), and the concentration was 1 mM.[34]

Fly Stocks and Rearing

The w^{1118} (#5905) flies were obtained from the Bloomington *Drosophila* stock center (BDSC; Indiana, United States). Fruit flies were cultured in common corn agar medium as reported in previous studies.[35] 3–5 day old female flies were used for experiments.

Table 1 List of Small Molecule in This Study

Name	Stock	Solvent	Production Lot
Resveratrol	1 mM	Ethanol	M17HB178543
Magnolol	1 mM	Ethanol	J22HB189358
Caffeic acid	1 mM	Ethanol	M28HB183194
Ferulic acid	1 mM	Ethanol	G13S11L124423
Kaempferol	1 mM	Ethanol	J17IB218678
Salidroside	1 mM	Ethanol	O14IB228927
Curcumin	1 mM	Ethanol	O21IB229303
Isoliquiritigenin	1 mM	Ethanol	S26GB162199
Baicalein	1 mM	Ethanol	A07IB222187
Quercetin	1 mM	Ethanol	O29HB199514

Model Construction

3–5 day old w^{1118} female flies were randomly assigned to control, model, DBD treatment (2.5, 10 and 40 mg/mL, respectively), and active ingredient treatment. Prior to model establishment, the groups were trained in an environment with 21% oxygen for 3 days, with normal food in the control and model groups, and drug-containing basal food in the DBD-dosed and active ingredient group. During experiment, flies in the control group were maintained in 21% oxygen, and flies in the model, DBD and active ingredient administration groups were maintained in the 2% oxygen environment. The control and model groups were incubated with normal food, and the DBD and active ingredient administered groups were incubated with drug-containing food.

Sleep Assay

The *Drosophila* activity monitoring system (DAMS) was used to quantify the sleep quality in flies as described previously.[36,37] The DAMS includes two sleep monitoring panels connecting the gas inlet and outlet pipelines. The first sleep monitor was used to monitor the impact of normoxia, while the second monitor was used to monitor the effect of hypoxia stress. When flies pass through the middle of the sleep monitoring panel, they are scanned by infrared radiation, and the system records their movements per minute. When flies were not scanned by infrared light within 5 min, it is defined as sleeping.[38]

Flies were trained under the appropriate conditions for the first 2 d. Flies were then transferred to the DAMS system on the third day without any recorded data. The first sleep monitor was maintained at 21% oxygen and was used to record data from the control group, and the second sleep monitor was maintained at 21% oxygen and was used to record data from the model, DBD treatment, and the active ingredient group on the fourth day. The sleep and movement related physiological indicators of flies were analyzed using ShinyR DAM.

Screening of Potential Targets for AHISD

To determine the pathogenesis of AHISD, key proteins were collected by searching the GEO database (https://www.ncbi.nlm.nih.gov/geo/) and the GeneCards database (https://www.genecards.org/). Clinical samples exhibiting symptoms of acute mountain sickness are defined as AHISD. The clinical dataset (GEO accession ID: GSE75665) includes five individuals with symptoms of acute mountain sickness and five healthy control individuals, sequenced using the Illumina HiSeq2000 sequencing platform (Homo sapiens). Meanwhile, human-related disease-causing genes were searched in GeneCards using the keyword “acute high-altitude induced sleep disorders”. Homologous genes between humans and fruit flies are converted online by g:Profiler (https://biit.cs.ut.ee/gprofiler/orth).

Transcriptome Analysis

Total RNA of the fly brains was extracted using the TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer’s protocol. The RNA library was constructed and sequenced. Firstly, RNA sequencing results were normalized.

Differential genes with p values < 0.05 and $|\text{Fold change}| > 1.2$ were then identified using DESeq2 analysis. GO function and KEGG pathway enrichment analysis were performed. The correlation analysis plot, volcano plot, GO, and KEGG enrichment results were all plotted online via the chiplot website (https://www.chiplot.online/#Sankey-plot). The transcriptome sequencing experiment was repeated three times for biological replication.

Analysis of Neurotransmitter Content

Firstly, take an appropriate amount of neurotransmitter standard (Table S1), prepare a single standard masterbatch with methanol or water, dilute it to a suitable concentration, and make a working standard solution. Approximately 500 fly brains from each group were placed into a centrifuge tube, and a certain amount of formic acid methanol solution was added to perform grinding and centrifugation. About 100 µL of the supernatant was taken and set as a low concentration substance detection sample and a high concentration detection sample. Finally, the samples of different groups were scanned and detected by multiple reaction monitoring, and the concentrations of neurotransmitters were calculated according to the standard curve (Table S2). The chromatography-mass spectrometry conditions were as follows: chromatographic column: ACQUITY UPLC$^{®}$ HSS T3 (5 µL injection, 40°C); mobile phases: A-0.1% formic acid in water, B-0.1% formic acid in methanol, gradient: 0–2 min 5% B → 6 min 50% B → 7 min 95% B → 10.1 min 5% B (flow rate of 0.25 mL/min); mass spectrometry: ESI source (± ion mode), ion source 500°C/4500V; air curtain gas 30psi, nebulisation/assist gas 50psi.

RT-qPCR Analysis

The total RNA of brains was extracted with Trizol reagent (Invitrogen) and then reverse transcribed for cDNA using Hifair$^{®}$III 1st Strand cDNA Synthesis SuperMix (Yeasen, China). Quantitative PCR was performed using the GFX 96 ConnectTM Optical Module (Bio-Rad Laboratories) and Hifair$^{®}$qPCR SYBR$^{®}$Green Master Mix (No Rox) (Yeasen, China). The $2^{-\Delta\Delta Ct}$ method was used for analysis with rp49 as the reference gene. Primer sequences were listed in Table 2.

Component Identification Analysis of DBD

The active ingredients of DBD were identified by Ultra Performance Liquid Chromatography -MS/MS (UPLC-MS/MS). In brief, spectral databases such as the public databases HMDB, LipidMaps, KEGG, ChEBI and standard library of Nomi metabolism were used to characterize the raw data, and the parameters were then set to ppm < 30 to obtain the compositional qualitative results.

DBD ingredients were screened using the swissADME web tool (http://www.swissadme.ch/index.php) based on the UPLC-MS/MS results. In brief, the first step was to find the active ingredients SMILES number in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), which was subsequently loaded into the swissADME web tool to evaluate the key parameters of the active ingredients online. The ingredients with good solubility, good gastrointestinal absorption, and

Table 2 List of Forward and Reverse Primers

Genes	Forward	Reverse
5HT-1A	TTCGTGGCCTGCCTAGTAAT	CCAGTAACGATCGACGGCAA
5HT-1B	ATTTCGCCAGTTTGGCCATT	CGTTGCTGGTGCGATAATCA
GS1	TGCGTCTGCTGCGTACTGGC	CGGCGTTTCCAGGTTGCGGTA
GS2	GGATGGCCCGTTTCCTCT	ACCAGCACCGTTCCAATC
Toll-1	GTGAGGTCGACAGGGTTCAG	TGAGACGGCGAGTGGTAAAC
Myd88	GGCTCGTTCCCTACACGATC	GAATGCTGGGAGTGGTCACC
Dif	ATGTTTGAGGAGGCTTTCGG	GAACCGGCGGTGCGACCCTCGC
PGRP-LC	AAACGATCCGTTGACTGGAC	TACGCTTGGATTCCGTTTTC
Imd	TTCGGCTCCGTCTACAACTT	GTGATCGATTATGGCCTGGT
RP49	CTTCATCCGCCACCAGTC	GCACCAGGAACTTCTTGAATC

high bioavailability were selected as the research objects. The targets of active compounds were predicted using TCMSP, PharmMapper, and the interaction pathways were analyzed using David databases.

Molecular Docking

Molecular docking was used to predict the binding affinity and conformation of active compounds to TLR4 or 5-HT1A. The sdf format file of active compounds and receptor protein structures were downloaded from the PubChem database and the RCSB PDB database (https://www.rcsb.org/), respectively. Charge distribution optimisation and rotatable bond parameters were set for the active components using AutoDockTools 1.5.6 software, and water molecules and ligands carried by the proteins were removed using PyMOL software. The AutoDock Tools 1.5.6 software was then used to complement the protein hydrogen atoms, assign molecular charges and set the grid box parameters for the docking region. Finally, molecular docking of compound-target was performed by Autodock Vina 1.1.2 (http://vina.scripps.edu/) and visualized by Discovery Studio software.

Statistical Analysis

Each experiment was repeated at least three biological replicates. The GraphPad Prism 7.03 was performed to analyze the data. Data were expressed as mean ± S.E.M. Normality and lognormality test were used to check whether the samples conformed to the normal distribution, and Wilcoxon signed-rank test was used if they did not. One-way ANOVA followed by Tukey's multiple comparison test was used to analyze difference among three and more groups. The difference between two groups were analyzed using an unpaired *t*-test. The significance level is determined by $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, respectively.

Results

DBD Supplementation Improves Sleep in AHISD Flies

To investigate the sleep-protective effects of DBD, the sleep-related indicators of females were determined. The modeling conditions for each group are shown in Figure 1A. The results showed that acute hypoxia could induce sleep disorders in flies, while administration of DBD improved the sleep duration of flies during a 24-h baseline period (Figure 1B). Meanwhile, DBD supplementation could restore sleep time during the day, night, and total sleep in a dose-dependent manner (Figure 1C–E). Together, these findings suggest that DBD exerts a sleep-improvement effect in AHISD flies.

AHISD Shares Conserved Pathogenesis Between Fruit Fly and Human

To further explore the conservative pathogenesis of AHISD, the combination of the clinical dataset, disease database and transcriptomics was employed. Firstly, the gene expression profiles between groups were consistent and exhibited minimal fluctuations, as shown in the gene box plot (Figure 2A). It was found that 921 upregulated-genes and 595 downregulated-genes based on the analysis of 14406 genes (Figure 2B). After comparative analysis of the clinical dataset, disease database and transcriptomes, it was revealed that there were 1252 core conserved genes (Figure 2C), and some of the conserved gene information was shown in Table 3. Then, the enrichment analysis of these core genes was conducted, and the results showed that it is mainly related to neuroactive ligand-receptor interaction, Toll and Imd signaling pathway, autophagy and mTOR signaling pathway (Figure 2D and E). These results suggest that multiple pathways in AHISD are conserved in fruit flies.

DBD Supplementation Regulates Various Gene Expression in AHISD Flies

To explore the mitigation mechanism of DBD, the gene expression of AHISD flies after DBD supplementation was dissected. The correlation coefficient plots indicated a high degree of within-group similarity and significant between-group differences (Figure 3A). Upon comparing the analysis of differences among three groups (|Fold change| > 1.2, $p < 0.05$), it was revealed that AHISD group had 175 up-regulated genes and 26 down-regulated genes, which was restored after DBD administration (Table S3, Figures S1 and 3B), and the major differential genes were shown in Figure 3C. Enrichment

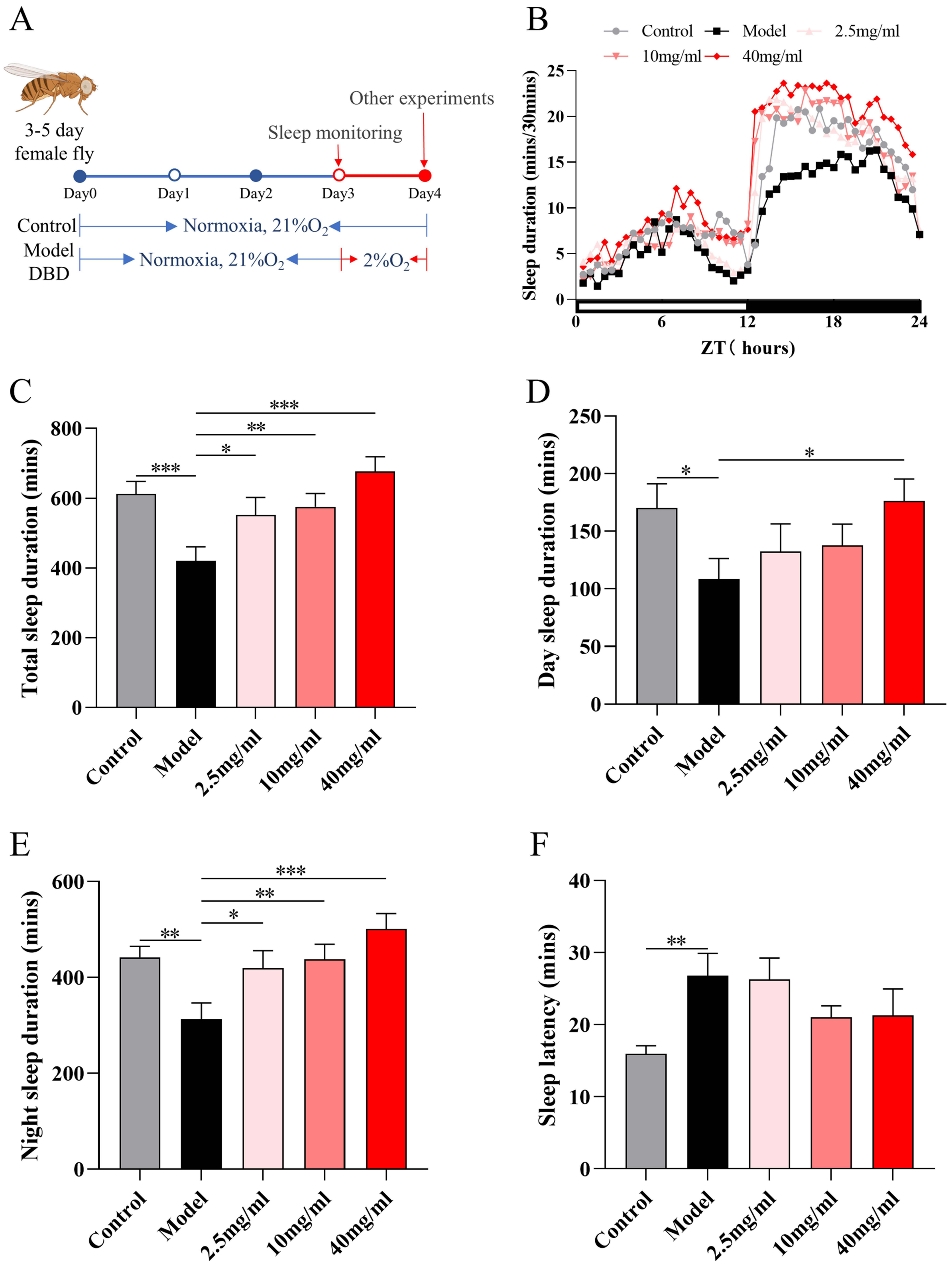

Figure 1 DBD alleviates sleep disorder in fruit flies under acute hypoxia. (**A**) Experimental flowchart of acute hypoxia modeling and DBD treatment in flies. Sleep status was evaluated using (**B**) sleep profiles in a 12 h light/12 h dark (LD) cycle, (**C**) total sleep duration, (**D**) day sleep duration, (**E**) night sleep duration and (**F**) sleep latency (n = 32-35). The data is expressed as mean ± SEM. Compared to model group, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ indicate significant difference.

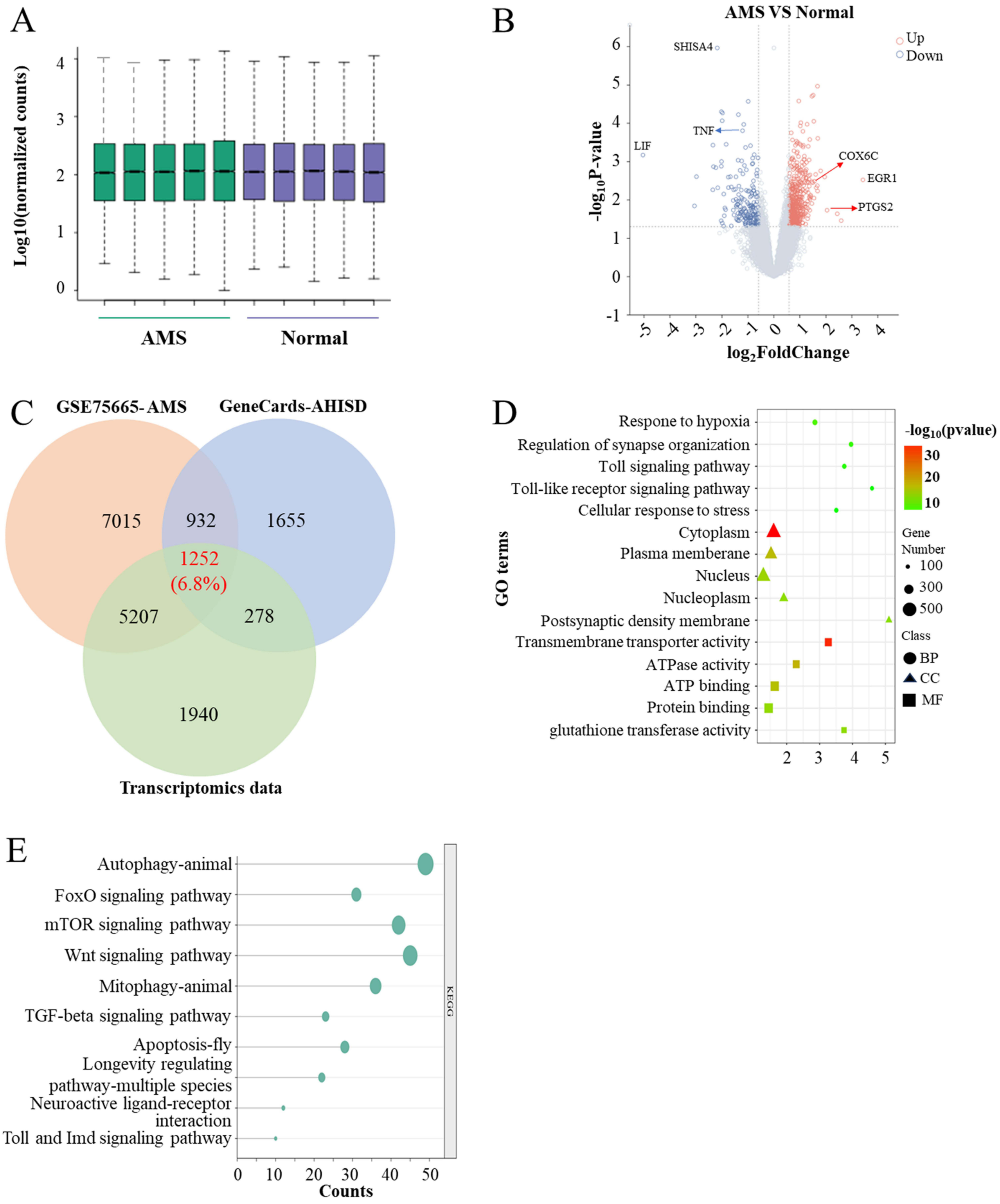

Figure 2 AHISD has conserved pathogenesis between fruit flies and humans. (**A**) Gene expression profiles of AMS and normal samples in clinical datasets. (**B**) The volcano plot of differently expressed genes. (**C**) Venny analysis of clinical datasets, disease databases, and transcriptomes revealed 1252 core conserved genes. (**D**) Enrichment analysis of core conserved gene. (**E**) KEGG pathway analysis of core conserved gene.

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Table 3 List of Top 20 Conserved Genes

Gene name (*Drosophila*)	Orthologs (Homo sapiens)	Pathways
Pten	PTEN	Autophagy - animal
Pi3K59F	PIK3C3	Autophagy - animal
Ras85D	HRAS	FoxO signaling pathway
Atg8b	GABARAP	FoxO signaling pathway
eIF4E6	EIF4E1B	mTOR signaling pathway
Mnd	SLC7A5	mTOR signaling pathway
CG34404	MCC	Wnt signaling pathway
Slmb	BTRC	Wnt signaling pathway
CG11700	UBB	Mitophagy - animal
Sima	HIF1A	Mitophagy - animal
Vis	TGIF2	TGF-beta signaling pathway
HDAC4	HDAC1	TGF-beta signaling pathway
Cyt-c-p	CYCS	Apoptosis - fly
Decay	CASP3	Apoptosis - fly
Chico	IRS1	Longevity regulating pathway - multiple species
S6K	RPS6KB1	Longevity regulating pathway - multiple species
GABA-B-R1	GABBR1	Neuroactive ligand-receptor interaction
GS1	GLUL	Neuroactive ligand-receptor interaction
Relish	NFKB1	Toll and Imd signaling pathway
Imd	RIPK1	Toll and Imd signaling pathway

analysis revealed that DBD supplementation regulated metabolic-related pathway, Toll and Imd signaling pathway, and neuroactive ligand-receptor interactions (Figure 3D and E). Therefore, these results suggest that DBD may exert protective effects against AHISD via multiple pathways.

DBD Supplementation Restores Acute Hypoxia-Induced Neurological Dysfunction in AHISD Flies

To further clarify the mechanism of DBD against AHISD, neural function indicators were tested. Liquid chromatography-mass spectrometry was used to detect neurotransmitter content, and the relevant information was shown in Figure S2. The PCA results showed stable intra group repeatability and significant inter group differences (Figure 4A). Acute hypoxia could lead to abnormal expressions of neurotransmitters in fly brain tissue (Figure 4B). After DBD intervention, the relative metabolic levels of Glutamine (Gln) and Glutamic acid (Glu) were restored (Figure 4C and D). The standard curve information of neurotransmitters was shown in Table S2. In addition, acute hypoxia down-regulated the mRNA levels of *5-HT1A, 5-HT1B, glutamine synthetase* 1 (*GS1*) and *glutamine synthetase* 2 (*GS2*), and up-regulated the gene expressions of *Toll-1, Myd88, Dif, PGRP-LC*, and *Imd*, while DBD administration could restore these gene expression levels (Figure 4E and F). Therefore, these results demonstrate that DBD exerts neuroprotective effects against AHISD.

Three Bioactive Compounds of DBD Improve Sleep Disorder in AHISD Flies

To investigate the bioactive compounds of DBD extract against AHISD, firstly UPLC-MS/MS was performed. The results showed that 19 compounds were identified from DBD extracts, as detailed in Table S4. It is anticipated that 10 compounds may alleviate sleep disorders according to the literature, and the solubility, GI absorption, and bioavailability score that predicted based on their chemical structures (Figure 5A–C). To further detect the efficacy of these 10 compounds, sleep index tests were conducted. Resveratrol, magnolol and caffeic acid could significantly improve total sleep time and nighttime sleep duration in AHISD flies (Figure 6A–C). Resveratrol and caffeic acid could prolong sleep duration (Figure 6D), while caffeic acid shortened sleep latency (Figure 6E).

The targets of resveratrol, magnolol and caffeic acid were predicted using TCMSP and PharmMapper, and the interaction of targets between the three compounds and AHISD was then analyzed (Figure 7A). It was found that

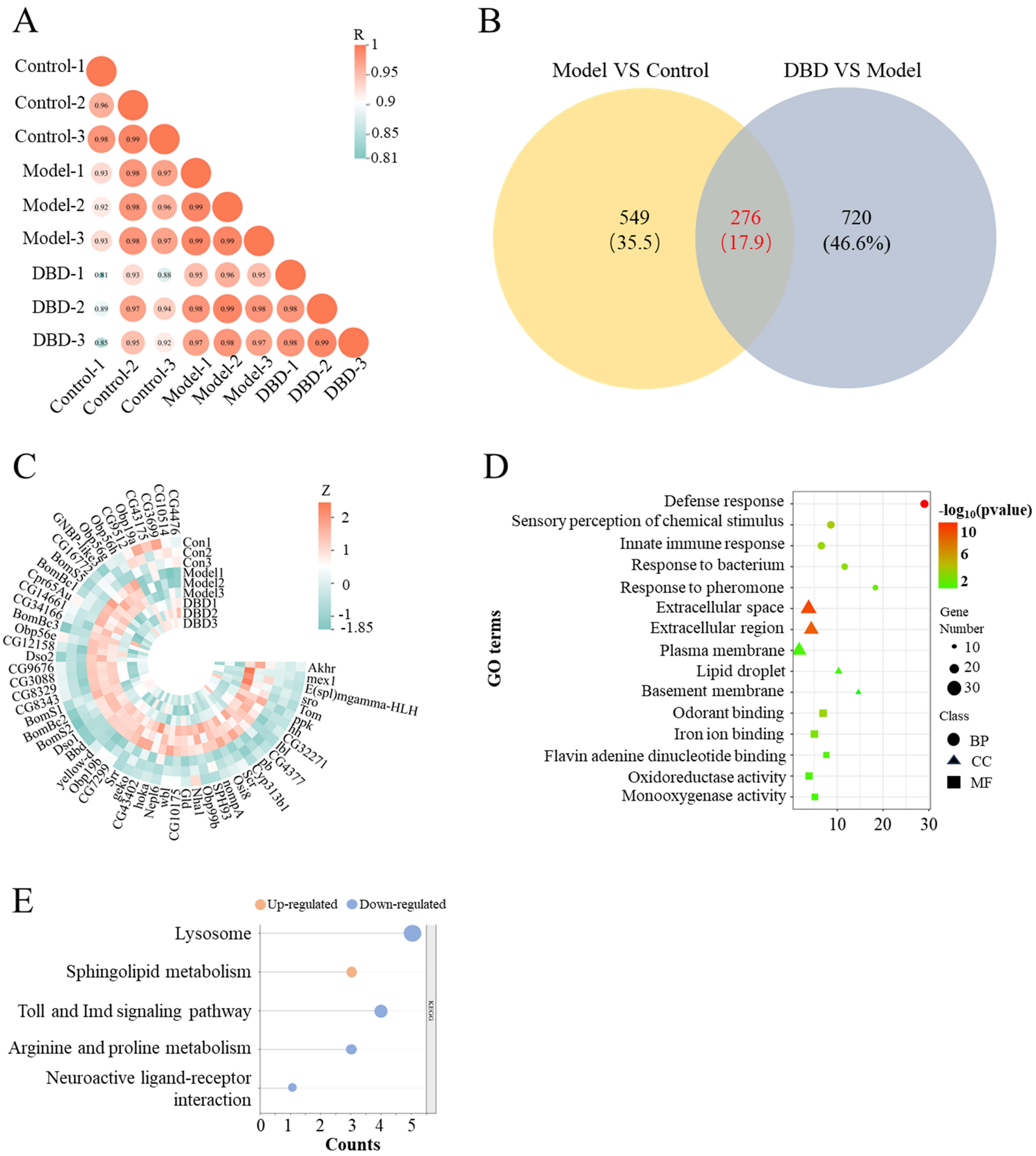

Figure 3 DBD regulates gene expression in AHISD flies. (**A**) The correlation coefficient chart. (**B**) Venny analysis among control, model, and DBD. (**C**) Cluster heatmap of core DEGs. (**D**) Enrichment analysis of core DEGs. (**E**) KEGG pathway analysis of core DEGs.

resveratrol, magnolol and caffeic acid could intervene in AHISD mainly via Toll-like signaling pathway and serotonergic synapse, respectively (Figure 7B–D). Molecular docking simulations were conducted between three compounds and receptor proteins, which showed that the docking affinity was less than −5.0 kcal/mol (Table 4) and the binding conformation was stable (Figure 7E and F). Therefore, these findings indicate that resveratrol, magnolol and caffeic acid are the main compounds of DBD against AHISD.

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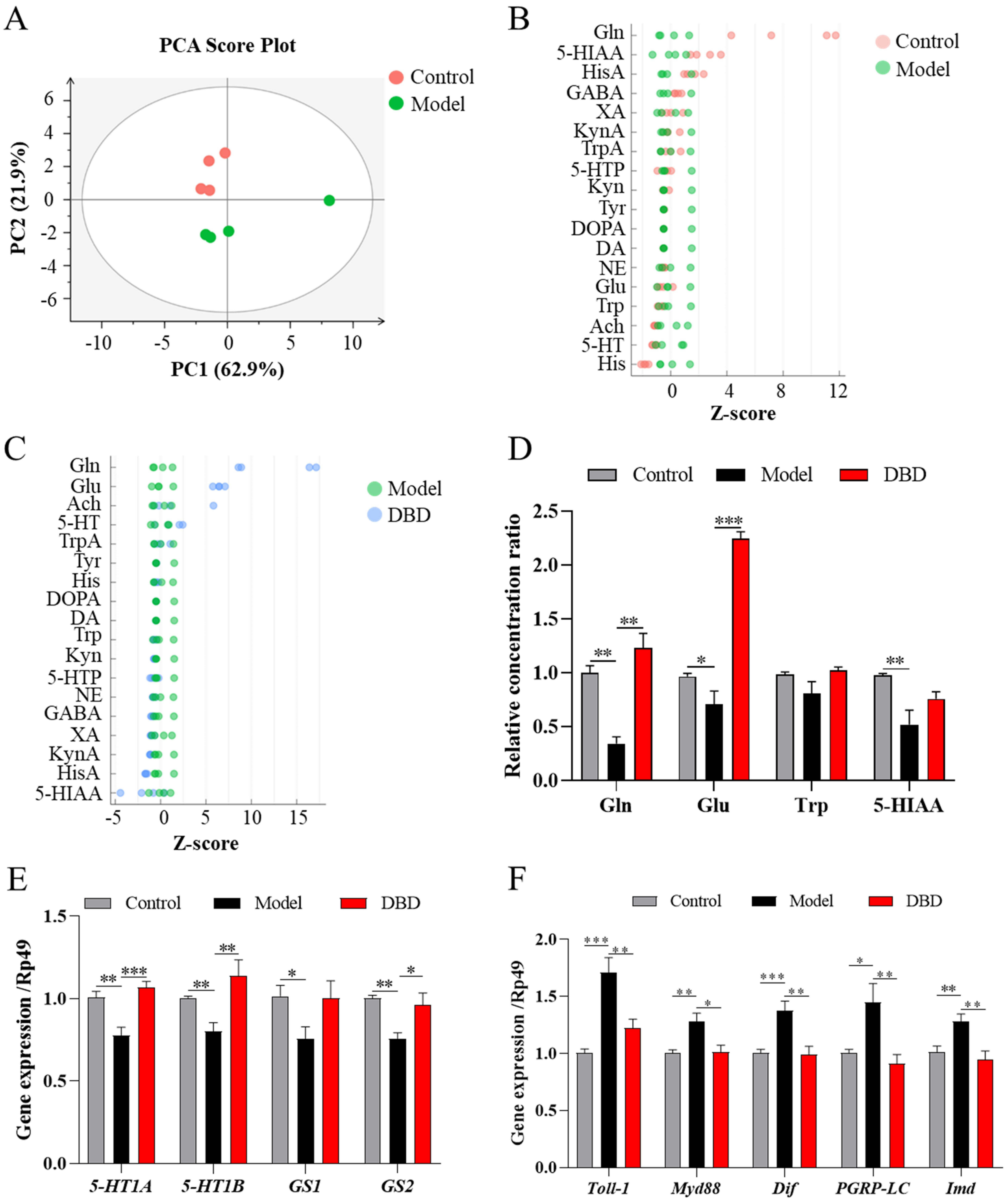

Figure 4 DBD exerts neuroprotective function in AHISD flies. (**A**) Principal Component Analysis. (**B** and **C**) Relative expression levels of neurotransmitters in different group. (**D**) The relative concentration of Gln, Glu, Trp, and 5-HIAA (n = 3). (**E**) The gene expression of neuroactive ligand-receptor interaction pathway (n = 3). (**F**) The gene expression of Toll and Imd signaling pathway (n = 3). The data is expressed as mean ± SEM. Compared to model group, **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 indicate significant difference.

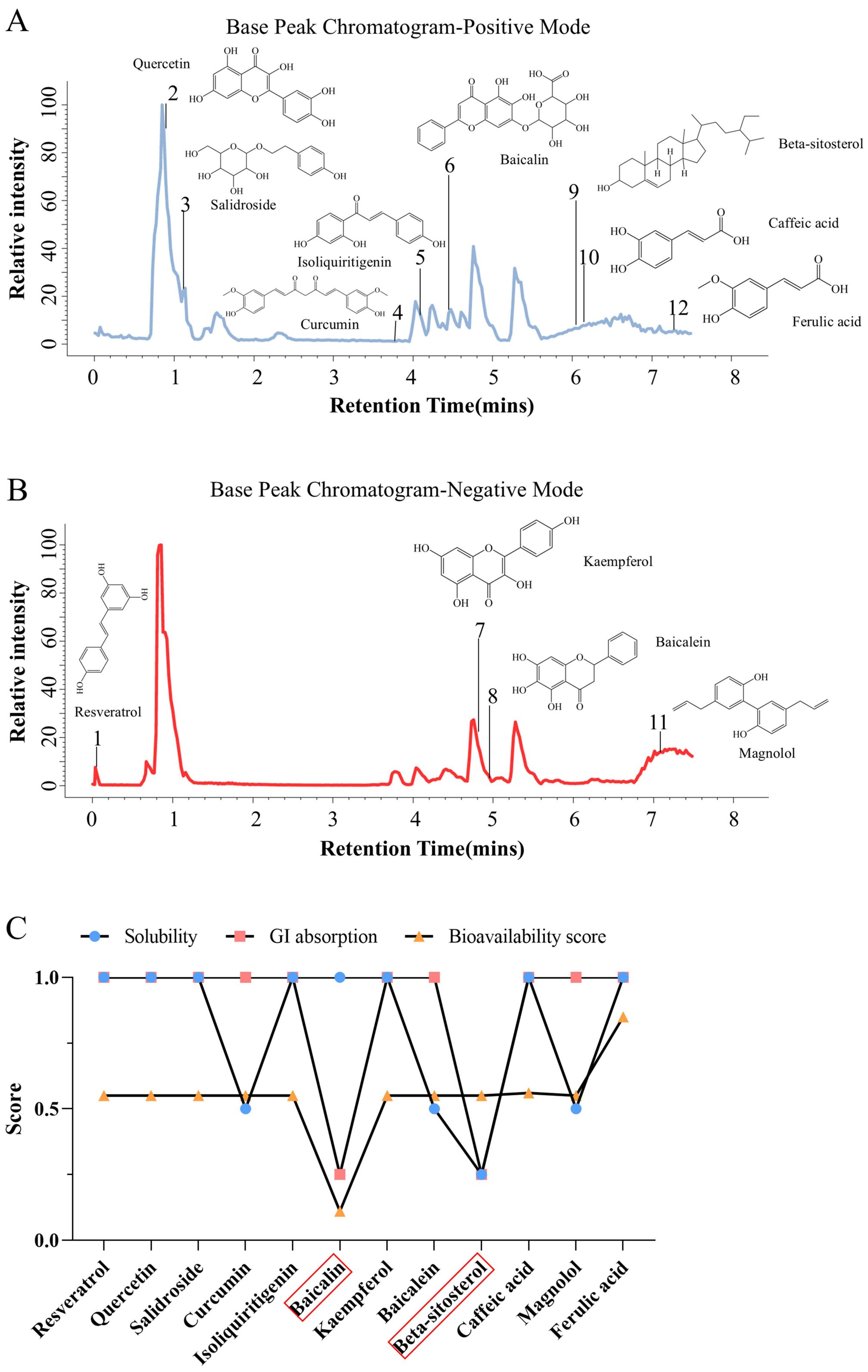

Figure 5 Characteristics of bioactive components in DBD. Identification of DBD base peak chromatogram positive (**A**) and negative modes (**B**) using UPLC-MS/MS. (**C**) The compound solubility, GI absorption, and bioavailability scores were predicted based on chemical structure.

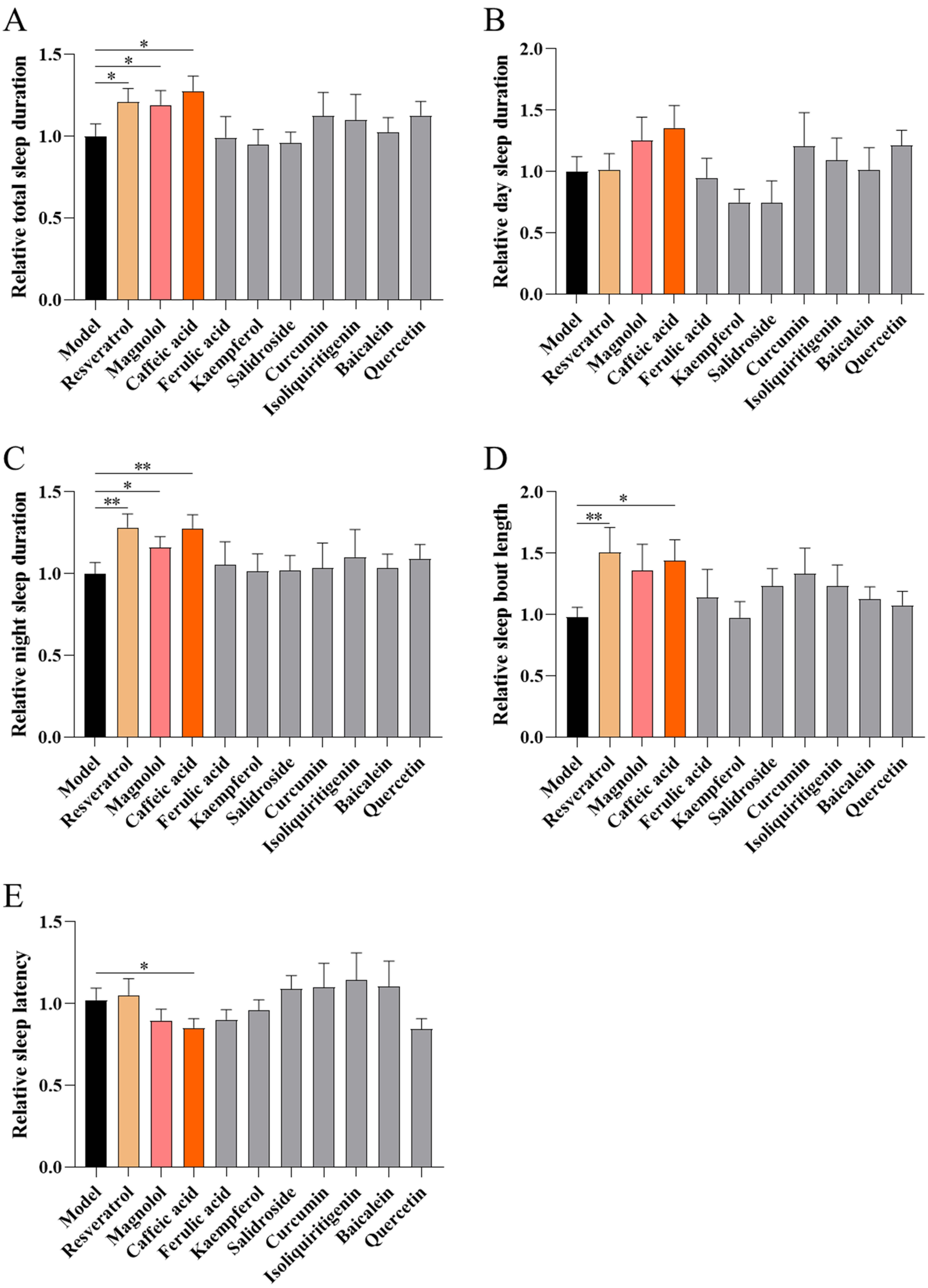

Figure 6 Resveratrol, magnolol and caffeic acid improved sleep disorder in AHISD flies. Sleep Status was evaluated using (**A**) relative total sleep duration, (**B**) relative day sleep duration, (**C**) relative night sleep duration, (**D**) relative sleep bout length and (**E**) relative sleep latency (n = 12-36). The data is expressed as mean ± SEM. Compared to model group, **p* < 0.05 and ***p* < 0.01 indicate significant difference.

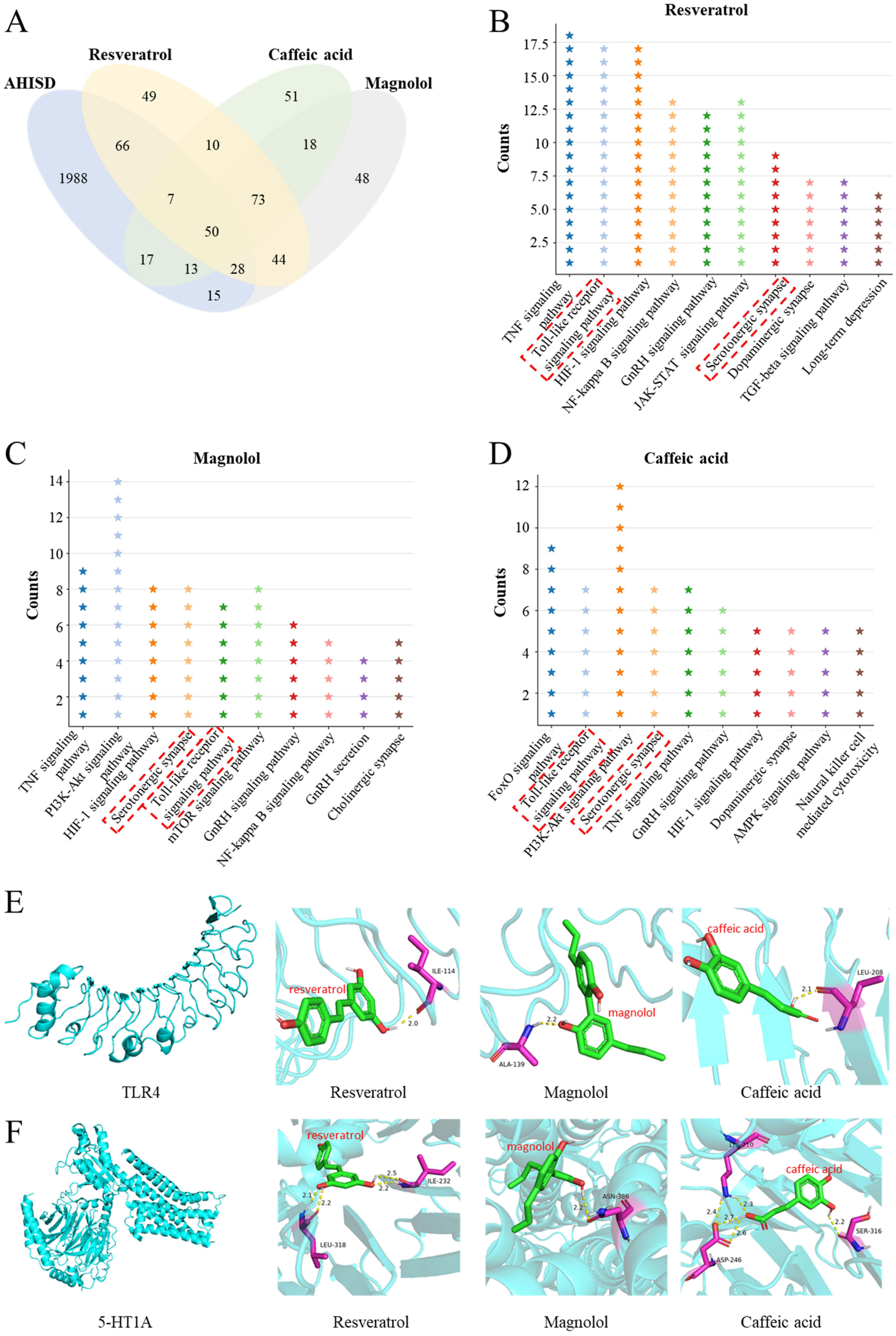

Figure 7 Resveratrol, magnolol, and caffeic acid intervened in AHISD through multiple pathways. (**A**) Venny diagram of interaction targets between compounds and AHISD. KEGG pathway analysis of interaction targets between resveratrol (**B**), magnolol (**C**), caffeic acid (**D**) and AHISD were analyzed. Molecular docking simulations were conducted between TLR4 (**E**), 5-HT1A (**F**) and compounds.

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Table 4 Docking Scores of Compounds and Receptor Proteins

Gene Name	PDB ID	Compound	Affinity (kcal/mol)
TLR4	2Z62	Resveratrol	−6.0
		Magnolol	−6.9
		Caffeic acid	−5.9
5HT-1A	8FYX	Resveratrol	−7.7
		Magnolol	−8.0
		Caffeic acid	−7.0

Discussion

Sleep plays an important role in maintaining people's normal physiological activities. AHISD as a plateau complication is commonly found in the high-altitude population,[39] causing the learning and memory impairments,[40] depression[41,42] and acute cerebral edema.[43] In this study, we investigated the sleep improvement effect and material basis of DBD in AHISD flies. The results indicated that DBD could enhance sleep duration and structure by restoring neural function homeostasis and the alleviation of neuroinflammation. Furthermore, resveratrol, magnolol and caffeic acid in DBD were identified to improve sleep in AHISD flies.

Currently, AHISD researches mainly focus on neurological dysfunction, including imbalanced neurotransmitters and related receptors levels, as well as neuroinflammation.[5,6] Our results showed that DBD could restore the decreased levels of Gln, Glu, 5-hydroxyindoleacetic acid (5-HIAA), 5-HT1A, 5-HT1B and GS in the brain of AHISD flies. Sleep-regulating neurotransmitters are associated with Gln and 5-HT metabolites, such as 5-HIAA. Gln could produce Glu that released into the synaptic cleft to bind with receptors,[44] while the remaining Glu is transported to glial cells and converted to Gln by GS, avoiding neurotoxicity caused by Glu accumulation.[45] Research has shown that sleep disorders induced by PCPA in rats were associated with a reduction in Gln content;[46] supplementation with Gln content could significantly increase sleep duration.[47] It was reported that high-altitude hypoxic induced the Gln content in rat serum.[48] 5-HIAA as the main metabolite of 5-HT could indirectly affect the function of 5-HT1A.[49,50] Studies have found that the decreases in 5-HIAA and 5-HT1A levels could induce sleep disorders.[51,52] In addition, under simulated hypoxic condition, the content of 5-HIAA in the brain decreased,[52] and 5-HT1A was disrupted.[53,54] These findings suggested that DBD could alleviate the imbalance of neurotransmitters and receptors induced by acute hypoxia.

Neuroinflammation also plays an important role in AHISD. TLR4 could activate the downstream NF-κB signaling pathway via the MyD88 pathway, promoting the expression of pro-inflammatory cytokine genes, and causing neuronal damage, and ultimately leads to sleep disorders.[55–57] TLR4 disrupts normal brain activity and alters sleep structure through interleukin-6 and osteopontin.[58] Furthermore, acute hypoxia exposure may activate the TLR4 pathway, leading to brain dysfunction and inducing sleep rhythm disorders.[59,60] We found that acute hypoxia exposure rapidly activated the innate immune pathways (Toll pathway: *Toll-1, Myd88, Dif*; Imd pathway: *PGRP-LC, Imd*) in fruit flies, which could be suppressed after DBD intervention. The results indicated that DBD could alleviate acute hypoxia induced neuroinflammatory response. It is important to point out that the existing evidence is only correlative, and in the future, we will further clarify the roles of Toll and Imd signaling pathway through inhibition or knockdown experiments.

Traditional Chinese medicine has complex ingredients, and its active ingredients work together to treat diseases such as insomnia.[61,62] DBD is composed of multiple components including terpenoids, flavonoids, phenolic acids, and gingerols.[63] Here, active ingredients of DBD were identified based on UPLC-MS/MS analysis, and the results showed that 10 ingredients had potential sleep improving effects. For example, resveratrol alleviated sleep disorders in APP fruit flies by regulating the expression of dSir2;[64] magnolol increased NREM and REM sleep time through GABA receptors;[65] caffeic acid had sedative effects in pentobarbital-induced sleeping time in mice.[66] Subsequently, the sleep monitoring experiment demonstrated that resveratrol, magnolol, and caffeic acid exhibit sleep-enhancing properties. In addition, using molecular docking, we found these three active ingredients bound to the corresponding receptor proteins in a stable conformation and all had better binding ability to 5-HT1A. These results suggested that the above three

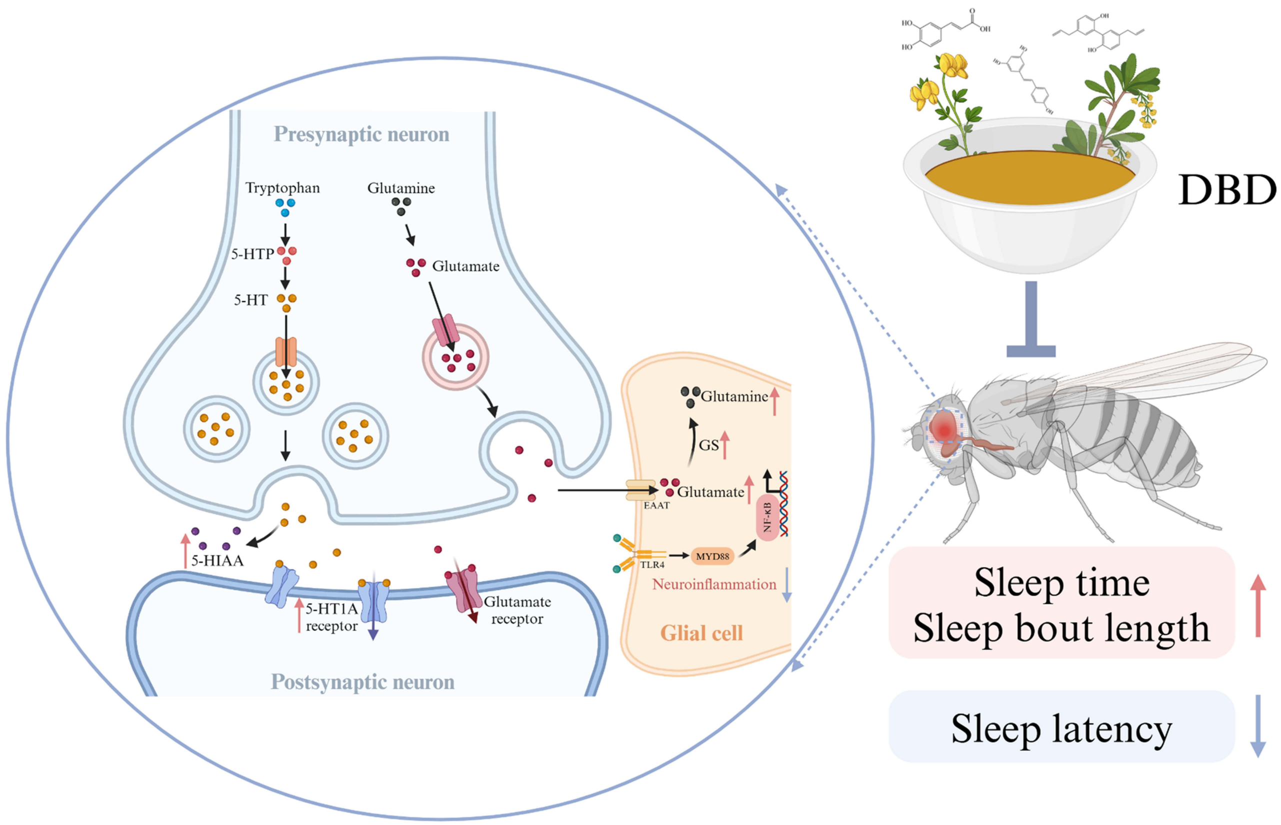

Figure 8 The protective effect and mechanism of DBD on acute hypoxia induced sleep disorders.

components may be the main active ingredients of DBD in alleviating AHISD. Notably, DBD is a complex mixture, the mechanism by which these three active ingredients work synergistically to alleviate AHISD requires further investigation.

In summary, this study used in vivo experiments and in-silico approaches to clarify the therapeutic mechanism and active ingredients of DBD against AHISD (Figure 8). Although the sleep improving effect of DBD still needs more experimental verification in clinical practice, it provides new ideas for the treatment of AHISD. The current results are only based on the *Drosophila* model, whereas mammals have more complex brain structures and sleep regulation mechanisms than the *Drosophila* model. For instance, although some neurotransmitters in fruit flies have the similar functions with these in mammals, including GABA, glutamate, acetylcholine, dopamine, 5-HT, and histamine, mammals differ from fruit flies in some neurotransmitter release sites and metabolic pathways.[67–69] Therefore, future studies will focus on the validation of mechanisms in mammals. In addition, *Drosophila* research tools could be applied to directly compare the function of specific neural circuits in mammals. By focusing on evolutionarily conserved mechanisms and integrating cross-species techniques, the translational value of *Drosophila* research could be maximised while circumventing the biases associated with model limitations. Due to the similarity in sleep structure and sleep related pathways between fruit flies and humans, AHISD flies are a perfect in vivo model for high-throughput drug screening.

Conclusion

DBD and its main active ingredients resveratrol, magnolol, and caffeic acid can improve AHISD, and its potential mechanism may be related to the restoration of neurological function.

Abbreviations

AHISD, Acute hypoxia induced sleep disorders; DBD, Danggui Buxue decoction.

Data Sharing Statement

The data that support the findings of this study are available on request from the corresponding author.

Ethical Statement

This study received exempt review approval from the Institutional Review Board (IRB) of Gansu University of Chinese Medicine, subject to the following prerequisites: (1) no deviation or modification of the study protocol may be made without prior written authorization from the IRB; and (2) any proposed modification of the principal investigator, source of funding, protocol, informed consent, questionnaires, or recruiting materials must be submitted to the IRB through a formal protocol modification application and be subject to reassessment.

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Disclosure

The authors declare that there are no conflicts of interest associated with this work.

References

1. Nussbaumer-Ochsner Y, Ursprung J, Siebenmann C, Maggiorini M, Bloch KE. Effect of short-term acclimatization to high altitude on sleep and nocturnal breathing. *Sleep*. 2012;35(3):419–423. doi:10.5665/sleep.1708
2. Heinzer R, Saugy JJ, Rupp T, et al. Comparison of sleep disorders between real and simulated 3450-m altitude. *Sleep*. 2016;39(8):1517–1523. doi:10.5665/sleep.6010
3. Tseng CH, Lin FC, Chao HS, Tsai HC, Shiao GM, Chang SC. Impact of rapid ascent to high altitude on sleep. *Sleep Breathing*. 2015;19(3):819–826. doi:10.1007/s11325-014-1093-7
4. Windsor JS, Rodway GW. Sleep disturbance at altitude. *Curr Opin Pulm Med*. 2012;18(6):554–560. doi:10.1097/MCP.0b013e328359129f
5. Ray K, Dutta A, Panjwani U, Thakur L, Anand JP, Kumar S. Hypobaric hypoxia modulates brain biogenic amines and disturbs sleep architecture. *Neurochem Int*. 2011;58(1):112–118. doi:10.1016/j.neuint.2010.11.003
6. Chen X, Zhang J, Lin Y, et al. Mechanism, prevention and treatment of cognitive impairment caused by high altitude exposure. *Front Physiol*. 2023;14:1191058. doi:10.3389/fphys.2023.1191058
7. Holst SC, Landolt HP. Sleep-wake neurochemistry. *Sleep Med clin*. 2022;17(2):151–160. doi:10.1016/j.jsmc.2022.03.002
8. Lemos Vde A, Dos Santos RV, Lira FS, Rodrigues B, Tufik S, de Mello MT. Can high altitude influence cytokines and sleep? *Mediators Inflammation*. 2013;2013:279365. doi:10.1155/2013/279365
9. Lin HQ, Gong AG, Wang HY, et al. Danggui Buxue Tang (Astragali Radix and Angelicae Sinensis Radix) for menopausal symptoms: a review. *J Ethnopharmacol*. 2017;199:205–210. doi:10.1016/j.jep.2017.01.044
10. Wang WK, Zhou Y, Fan L, Sun Y, Ge F, Xue M. The antidepressant-like effects of Danggui Buxue Decoction in GK rats by activating CREB/BDNF/TrkB signaling pathway. *Phytomedicine*. 2021;89:153600. doi:10.1016/j.phymed.2021.153600
11. Dou Y, Wang Y, Shu Y, et al. Combination treatment of Danggui Buxue Decoction and endothelial progenitor cells can enhance angiogenesis in rats with focal cerebral ischemia and hyperlipidemia. *J Ethnopharmacol*. 2023;314:116563. doi:10.1016/j.jep.2023.116563
12. Yin B. The clinical study on the treatment of Parkinson's disease (qi deficiency syndrome) based on the theory of "essence and blood", Master's thesis, Chengdu University of TCM. 2018.
13. Lee YA, Kim YJ, Lee JS, Lee S, Goto Y. Imbalance between dopamine and serotonin caused by neonatal habenula lesion. *Behav Brain Res*. 2021;409:113316. doi:10.1016/j.bbr.2021.113316
14. Yao W, Zhang L, Hua Y, et al. The investigation of anti-inflammatory activity of volatile oil of Angelica sinensis by plasma metabolomics approach. *Int Immunopharmacol*. 2015;29(2):269–277. doi:10.1016/j.intimp.2015.11.006
15. Xu H, Zhang T, He L, Yuan M, Yuan X, Wang S. Exploring the mechanism of Danggui Buxue Decoction in regulating atherosclerotic disease network based on integrated pharmacological methods. *Biosci Rep*. 2021;41(10). doi:10.1042/bsr20211429
16. Kambe D, Kotani M, Yoshimoto M, Kaku S, Chaki S, Honda K. Effects of quercetin on the sleep-wake cycle in rats: involvement of gamma-aminobutyric acid receptor type A in regulation of rapid eye movement sleep. *Brain Res*. 2010;1330:83–88. doi:10.1016/j.brainres.2010.03.033

17. Liu WL, Wu BF, Shang JH, Wang XF, Zhao YL, Huang AX. Moringa oleifera seed ethanol extract and its active component kaempferol potentiate pentobarbital-induced sleeping behaviours in mice via a GABAergic mechanism. *Pharm Biol*. 2022;60(1):810–824. doi:10.1080/13880209.2022.2056207
18. Tu Y, Cheng SX, Sun HT, Ma TZ, Zhang S. Ferulic acid potentiates pentobarbital-induced sleep via the serotonergic system. *Neurosci lett*. 2012;525(2):95–99. doi:10.1016/j.neulet.2012.07.068
19. Li W, Wang Z, Syed S, et al. Chronic social isolation signals starvation and reduces sleep in Drosophila. *Nature*. 2021;597(7875):239–244. doi:10.1038/s41586-021-03837-0
20. Knapp EM, Kaiser A, Arnold RC, et al. Mutation of the Drosophila melanogaster serotonin transporter dSERT impacts sleep, courtship, and feeding behaviors. *PLoS Genet*. 2022;18(11):e1010289. doi:10.1371/journal.pgen.1010289
21. Staats S, Lüersen K, Wagner AE, Rimbach G. Drosophila melanogaster as a versatile model organism in food and nutrition research. *J Agricult Food Chem*. 2018;66(15):3737–3753. doi:10.1021/acs.jafc.7b05900
22. Huber R, Hill SL, Holladay C, Biesiadecki M, Tononi G, Cirelli C. Sleep homeostasis in Drosophila melanogaster. *Sleep*. 2004;27(4):628–639. doi:10.1093/sleep/27.4.628
23. Lauss M, Kriegner A, Vierlinger K, Noehammer C. Characterization of the drugged human genome. *Pharmacogenomics*. 2007;8(8):1063–1073. doi:10.2217/14622416.8.8.1063
24. Ueno T, Tomita J, Tanimoto H, et al. Identification of a dopamine pathway that regulates sleep and arousal in Drosophila. *Nat Neurosci*. 2012;15(11):1516–1523. doi:10.1038/nn.3238
25. Yuan Q, Joiner WJ, Sehgal A. A sleep-promoting role for the Drosophila serotonin receptor 1A. *Current Biol*. 2006;16(11):1051–1062. doi:10.1016/j.cub.2006.04.032
26. Williams JA, Sathyanarayanan S, Hendricks JC, Sehgal A. Interaction between sleep and the immune response in Drosophila: a role for the NFkappaB relish. *Sleep*. 2007;30(4):389–400. doi:10.1093/sleep/30.4.389
27. Li QF, Wang H, Zheng L, et al. Effects of modest hypoxia and exercise on cardiac function, sleep-activity, negative geotaxis behavior of aged female drosophila. *Front Physiol*. 2019;10:1610. doi:10.3389/fphys.2019.01610
28. Ping X, Li Q, Ding M, et al. Effects of hypoxic compound exercise to promote HIF-1α expression on cardiac pumping function, sleep activity behavior, and exercise capacity in Drosophila. *FASEB j*. 2024;38(5):e23499. doi:10.1096/fj.202302269R
29. He J, Han S, Wang Y, et al. Irinotecan cause the side effects on development and adult physiology, and induces intestinal damage via innate immune response and oxidative damage in Drosophila. *Biomed Pharmacothe*. 2023;169:115906. doi:10.1016/j.biopha.2023.115906
30. Liu H, Yang L, Wan C, et al. Exploring potential mechanism of ciwujia tablets for insomnia by UPLC-Q-TOF-MS/MS, network pharmacology, and experimental validation. *Front Pharmacol*. 2022;13:990996. doi:10.3389/fphar.2022.990996
31. Wang X, Zhang X, Li J, et al. Network pharmacology and LC-MS approachs to explore the active compounds and mechanisms of Yuanjiang decoction for treating bradyarrhythmia. *Comput Biol Med*. 2023;152:106435. doi:10.1016/j.compbiomed.2022.106435
32. Wang Y, Qin Y, Kang Q, et al. Therapeutic potential of Astragalus membranaceus-Pueraria lobata decoction for the treatment of chemotherapy bowel injury. *FASEB j*. 2024;38(19):e70102. doi:10.1096/fj.202401677R
33. He L, Zhang Y, Li J, et al. Dunhuang Dabupi Decoction and its active components alleviate ulcerative colitis by activating glutathione metabolism and inhibiting JAK-STAT pathway in Drosophila and mice. *J Ethnopharmacol*. 2025;346:119717. doi:10.1016/j.jep.2025.119717
34. Xiu M, Li B, He L, et al. Caffeic acid protects against ulcerative colitis via inhibiting mitochondrial apoptosis and immune overactivation in Drosophila. *Drug Des Devel Ther*. 2025;19:2157–2172. doi:10.2147/dddt.S499284
35. Tuo W, Wang S, Shi Y, et al. Angelica sinensis polysaccharide extends lifespan and ameliorates aging-related diseases via insulin and TOR signaling pathways, and antioxidant ability in Drosophila. *Int J Biol Macromol*. 2023;241:124639. doi:10.1016/j.ijbiomac.2023.124639
36. Li X, Yang S, Wang S, et al. Regulation and mechanism of Astragalus polysaccharide on ameliorating aging in Drosophila melanogaster. *Int J Biol Macromol*. 2023;234:123632. doi:10.1016/j.ijbiomac.2023.123632
37. Wang S, Zhou S, Jiang X, Yang D, He J, Xiu M. Acute hypoxia induces sleep disorders via sima/HIF-1α regulation of circadian rhythms in adult Drosophila. *Comparat biochem Physiol Toxicol Pharmacol*. 2025;294:110192. doi:10.1016/j.cbpc.2025.110192
38. Andretic R, Shaw PJ. Essentials of sleep recordings in Drosophila: moving beyond sleep time. *Methods Enzymol*. 2005;393:759–772. doi:10.1016/s0076-6879(05)93040-1
39. Tang XG, Zhang JH, Gao XB, et al. Sleep quality changes in insomniacs and non-insomniacs after acute altitude exposure and its relationship with acute mountain sickness. *Neuropsychiatr Dis Treat*. 2014;10:1423–1432. doi:10.2147/ndt.S67218
40. Roy K, Chauhan G, Kumari P, et al. Phosphorylated delta sleep inducing peptide restores spatial memory and p-CREB expression by improving sleep architecture at high altitude. *Life Sci*. 2018;209:282–290. doi:10.1016/j.lfs.2018.08.026
41. Jin Y, Li J, Ye J, et al. Mapping associations between anxiety and sleep problems among outpatients in high-altitude areas: a network analysis. *BMC Psychiatry*. 2023;23(1):341. doi:10.1186/s12888-023-04767-z
42. Wang Y, Guang Z, Zhang J, et al. Effect of sleep quality on anxiety and depression symptoms among college students in China's Xizang Region: the mediating effect of cognitive emotion regulation. *Behav Sci*. 2023;13(10):861. doi:10.3390/bs13100861
43. Zhou Y, Huang X, Zhao T, et al. Hypoxia augments LPS-induced inflammation and triggers high altitude cerebral edema in mice. *Brain Behav Immun*. 2017;64:266–275. doi:10.1016/j.bbi.2017.04.013
44. Zhang D, Hua Z, Li Z. The role of glutamate and glutamine metabolism and related transporters in nerve cells. *CNS Neurosci Ther*. 2024;30(2):e14617. doi:10.1111/cns.14617
45. Machado-Vieira R, Ibrahim L, Henter ID, Zarate CA Jr. Novel glutamatergic agents for major depressive disorder and bipolar disorder. *Pharmacol Biochem Behav*. 2012;100(4):678–687. doi:10.1016/j.pbb.2011.09.010
46. Qiao T, Wang Y, Liang K, et al. Effects of the Radix Ginseng and Semen Ziziphi Spinosae drug pair on the GLU/GABA-GLN metabolic cycle and the intestinal microflora of insomniac rats based on the brain-gut axis. *Front Pharmacol*. 2022;13:1094507. doi:10.3389/fphar.2022.1094507
47. Nakagawa H, Nakane S, Ban G, Tomita J, Kume K. Effects of D-amino acids on sleep in Drosophila. *Biochem Biophys Res Commun*. 2022;589:180–185. doi:10.1016/j.bbrc.2021.11.107
48. Koundal S, Khushu S, Gandhi S. Studies on metabolic alterations due to hypobaric hypoxia in serum using NMR spectroscopy. *Biomarkers*. 2022;27(6):562–567. doi:10.1080/1354750x.2022.2076152

49. Saeed R, Mahmood K, Ali SB, Haleem DJ. Behavioral, hormonal, and serotonergic responses to different restricted feeding schedules in rats. *Int J Tryptophan Res*. 2022;15:11786469221104729. doi:10.1177/11786469221104729
50. Duan D, Tu Y, Yang X, Liu P. Electroacupuncture Restores 5-HT System Deficit in Chronic Mild Stress-Induced Depressed Rats. *Evidence-Based Complementary Alternative Med*. 2016;2016:7950635. doi:10.1155/2016/7950635
51. Chojnacki C, Gąsiorowska A, Popławski T, et al. Beneficial effect of increased tryptophan intake on its metabolism and mental state of the elderly. *Nutrients*. 2023;15(4):847. doi:10.3390/nu15040847
52. Trouvin JH, Prioux-Guyonneau M, Cohen Y, Jacquot C. Rat brain monoamine metabolism and hypobaric hypoxia: a new approach. *Gen Pharmacol*. 1986;17(1):69–73. doi:10.1016/0306-3623(86)90013-3
53. Lee KKY, Chattopadhyaya B, Do Nascimento ASF, et al. Neonatal hypoxia impairs serotonin release and cognitive functions in adult mice. *Neurobiol Dis*. 2024;193:106465. doi:10.1016/j.nbd.2024.106465
54. Mikhailenko VA, Butkevich IP. The role of 5-HT1A receptors in long-term adaptation of newborn rats to hypoxia. *Bull Exp Biol Med*. 2016;161(4):491–494. doi:10.1007/s10517-016-3445-8
55. Nighot M, Rawat M, Al-Sadi R, Castillo EF, Nighot P, Ma TY. Lipopolysaccharide-induced increase in intestinal permeability is mediated by TAK-1 activation of IKK and MLCK/MYLK gene. *Am J Pathol*. 2019;189(4):797–812. doi:10.1016/j.ajpath.2018.12.016
56. Zusso M, Lunardi V, Franceschini D, et al. Ciprofloxacin and levofloxacin attenuate microglia inflammatory response via TLR4/NF-kB pathway. *J Neuroinflammation*. 2019;16(1):148. doi:10.1186/s12974-019-1538-9
57. Liu B, Li F, Xu Y, Wu Q, Shi J. Gastrodin improves cognitive dysfunction in REM sleep-deprived rats by regulating TLR4/NF-κB and Wnt/β-Catenin signaling pathways. *Brain Sci*. 2023;13(2). doi:10.3390/brainsci13020179
58. Sartorius T, Lutz SZ, Hoene M, et al. Toll-like receptors 2 and 4 impair insulin-mediated brain activity by interleukin-6 and osteopontin and alter sleep architecture. *FASEB j*. 2012;26(5):1799–1809. doi:10.1096/fj.11-191023
59. Liu P, Pan L, Cui L, et al. Cordycepin ameliorates acute hypobaric hypoxia induced blood-brain barrier disruption, and cognitive impairment partly by suppressing the TLR4/NF-κB/MMP-9 pathway in the adult rats. *Eur J Pharmacol*. 2022;924:174952. doi:10.1016/j.ejphar.2022.174952
60. Liu S. Effect and mechanism of dexmedetomidine on sleep rhythm disturbance induced by hypobaric hypoxia in rats, Master's thesis, Jinzhou Medical University. 2023.
61. Li YM, Shen CY, Jiang JG. Sedative and hypnotic effects of the saponins from a traditional edible plant Liriope spicata Lour. in PCPA-induced insomnia mice. *J Ethnopharmacol*. 2024;327:118049. doi:10.1016/j.jep.2024.118049
62. Li R, Pan Y, Jing N, et al. Flavonoids from mulberry leaves exhibit sleep-improving effects via regulating GABA and 5-HT receptors. *J Ethnopharmacol*. 2025;337(Pt 3):118734. doi:10.1016/j.jep.2024.118734
63. Huang L, Liu Q, Zhang W, et al. Comprehensive quality evaluation of danggui-jianzhong decoction by fingerprint analysis, multi-component quantitation and UPLC-Q-TOF-MS. *J Chromatogr Sci*. 2024;62(7):635–648. doi:10.1093/chromsci/bmae034
64. Hao Y, Shao L, Hou J, et al. Resveratrol and Sir2 reverse sleep and memory defects induced by amyloid precursor protein. *Neurosci Bull*. 2023;39(7):1117–1130. doi:10.1007/s12264-023-01056-3
65. Chen CR, Zhou XZ, Luo YJ, Huang ZL, Urade Y, Qu WM. Magnolol, a major bioactive constituent of the bark of Magnolia officinalis, induces sleep via the benzodiazepine site of GABA(A) receptor in mice. *Neuropharmacology*. 2012;63(6):1191–1199. doi:10.1016/j.neuropharm.2012.06.031
66. Nugroho A, Kim MH, Choi J, et al. Phytochemical studies of the phenolic substances in Aster glehni extract and its sedative and anticonvulsant activity. *Arch Pharmacal Res*. 2012;35(3):423–430. doi:10.1007/s12272-012-0304-7
67. Lee D, Su H, O'Dowd DK. GABA receptors containing Rdl subunits mediate fast inhibitory synaptic transmission in Drosophila neurons. *J Neurosci*. 2003;23(11):4625–4634. doi:10.1523/jneurosci.23-11-04625.2003
68. Jan LY, Jan YN. L-glutamate as an excitatory transmitter at the Drosophila larval neuromuscular junction. *J Physiol*. 1976;262(1):215–236. doi:10.1113/jphysiol.1976.sp011593
69. Roeder T. Tyramine and octopamine: ruling behavior and metabolism. *Annual Rev Entomol*. 2005;50:447–477. doi:10.1146/annurev.ento.50.071803.130404