Cannabidiol Combats Depression Through Isgs-Mediated Inflammation in Zebrafish Model

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Abstract. Major depressive disorder (MDD) is chronic and disabling. Current monoaminergic drugs act slowly and fail to achieve remission in many patients. The endocannabinoid system (ECS), which regulates mood, reward processing, and synaptic plasticity, has emerged as a promising target, yet clinical results are inconsistent, and key determinants such as dose, cellular context, and individual variability remain unresolved. We addressed these gaps by coupling a systematic review of placebo-controlled trials of exogenous cannabinoids with an experimental zebrafish model of reserpine-induced depression. In zebrafish larvae, behavior was quantified in the light-dark box, and whole-organism RNA sequencing was used to map the mechanism. The result showed largely non-significant antidepressant outcomes, with efficacy appearing formulation-dependent. In zebrafish, cannabidiol (CBD) at the maximum tolerated concentration (0.312 μg/mL) partially rescued depression-like behavior. Transcriptomics analysis revealed selective upregulation of interferon-stimulated genes (ISGs), including *IFIT15* and *RSAD2*, with enrichment of immune, synaptic, and metabolic pathways. These cross-species findings indicate that CBD can ameliorate depressive phenotypes through endocannabinoid-linked modulation of neuroimmune pathways. They highlight dose sensitivity and microglial state as likely drivers of heterogeneity and motivate species-appropriate dosing and stratification by inflammatory status. Interferon-stimulated gene networks emerge as tractable entry points for developing safer, more effective, and personalized antidepressant strategies targeting the ECS.

Keywords: Zebrafish; Cannabidiol (CBD); Depression; Immune response; Reward system.

1. Introduction

Major depressive disorder (MDD) is a chronic, disabling mental illness characterized by low mood, loss of interest, and impaired functioning (American Psychiatric Association, 2013). With over 280 million people affected worldwide, it is the leading cause of disability, especially among women and adolescents (World Health Organization, 2023). Current first-line treatments primarily target monoaminergic systems, including selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs). However, nearly 50% of patients do not achieve remission after multiple trials, leading to the classification of treatment-resistant depression. This suggests the limited efficacy of traditional antidepressants for the majority of MDD patients, underscoring the urgent need for mechanistically distinct therapeutic approaches. Even when effective, these drugs often require weeks to take effect and are associated with adverse effects such as sexual dysfunction, weight gain, and emotional blunting (Chu & Wadhwa, 2023; McInnes & Marton, 2024). Recent research has shifted toward alternative pathways, including glutamatergic modulation (e.g., ketamine) (Silva et al., 2024) and reward system enhancement (Gonçalves et al., 2025). Among these, the endocannabinoid system (ECS) has gained attention for its role in regulating mood, stress response, and synaptic plasticity, presenting a promising target for novel antidepressant development.

Emerging evidence highlights the role of the reward system in motivation, pleasure, and reinforcement learning, particularly the mesolimbic dopamine pathway from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and prefrontal cortex (PFC). Dysfunction in this circuit is linked to anhedonia and motivational deficits in MDD (Höflich et al., 2018). Notably, the ECS modulates this reward pathway and plays a key role in mood regulation (Fattore et al., 2010). ECS includes endogenous ligands (anandamide, 2-AG), cannabinoid receptors (CB1 and CB2 receptors),

and metabolic enzymes like FAAH (Mechoulam & Parker, 2012). CB1 receptors are abundant in brain regions involved in emotion and reward, influencing dopaminergic signaling (Hill & Gorzalka, 2009). In rodents, ECS enhancement via CB1 agonists (e.g., Cannabinoid (CBD), Tetrahydro cannabinoid (THC)) leads to robust antidepressant-like effects, including reduced immobility in forced swim tests and behavioral recovery in chronic stress models (Hill & Gorzalka, 2005). CBD may enhance ECS tone and modulate neural circuits relevant to depression, with fewer psychoactive effects than THC. These findings position the ECS as a key interface between reward dysfunction and depressive pathophysiology, offering novel targets for intervention. By pharmacologically modulating the ECS, it may be possible to restore reward circuit integrity and alleviate depressive symptoms.

Although some clinical trials indicate the antidepressant potential of CBD, the reported efficacy is vastly different, even contradictory. For example, while CBD has significant effects on depressive symptoms manifested by subjects with mild to moderate anxiety (Gundugurti et al., 2024), it has limited effects on acute bipolar disorder (Pinto et al., 2023). Such a discrepancy may arise from the non-linear relationship between the concentration of medications and therapeutic effects. Besides, the complexity of the ECS modulation mechanism limits the translation of these findings to clinical practice. While CB1 agonists can enhance mood rapidly, they can also trigger adverse psychoactive effects (e.g., addiction), and CB1 antagonists can induce depressive-like behavior (Moreira et al., 2009). Furthermore, the interactions between the ECS and other nervous systems remain uncertain. Therefore, while ECS modulation holds potential, further research is needed to refine dosing strategies, safety protocols, and ECS mechanisms that maintain body balance by producing endocannabinoids that bind to CB1 and CB2 receptors, affecting functions like mood, pain, and appetite, before being broken down by enzymes.

In response to these limitations, this study promotes a research strategy focusing on the zebra fish as an animal model. Zebra fish and humans share a highly conserved ECS with CB1 receptors concentrating in the brain (Bailone et al., 2022). Besides, transparent larvae with abundant CB1 receptors and quick behavioral responses are suitable for high-content screening (Galindo-Villegas, 2020). By constructing a reserpine-induced depression-like model, combining it with exogenous CBD intervention, systematically evaluating the depressive-like phenotypic changes at the behavioral level, and using transcriptome analysis to reveal the neural regulatory network that activates ECS to treat depression, the study aims to address critical gaps in existing studies in cross-species verification and in-depth mechanism analysis. The findings may provide an empirical basis for the development of ECS-targeted antidepressant strategies with fewer side effects.

2. Method

2.1. Literature search strategy

Use the search terms including “cannabinoids” and “depression” in the PubMed database, and select the filter of “controlled clinical trial”, and include studies published up to 12 June 2025:

((“cannabinoids”[Supplementary Concept] OR “cannabinoids”[All Fields] OR “cannabinoid”[All Fields] OR “cannabinoids”[MeSH Terms]) AND (“depressed”[All Fields] OR “depression”[MeSH Terms] OR “depression”[All Fields] OR “depressions”[All Fields] OR “depression s”[All Fields] OR “depressive disorder”[MeSH Terms] OR (“depressive”[All Fields] AND “disorder”[All Fields]) OR “depressive disorder”[All Fields] OR “depressivity”[All Fields] OR “depressive”[All Fields] OR “depressively”[All Fields] OR “depressiveness”[All Fields] OR “depressives”[All Fields])) AND (controlledclinicaltrial[Filter])

2.2. Inclusion and exclusion criteria

2.2.1 Study inclusion criteria

This study focuses on placebo-controlled clinical trials involving patients diagnosed with depression (including those with comorbid conditions, provided depression is a measured outcome) and the use of cannabinoid-based compounds as potential therapeutic interventions.

2.2.2 Study exclusion criteria

This analysis excludes studies that: report depression as an adverse effect of CBD, include participants with substance abuse that may confound mood outcomes, or lack relevant outcome data.

2.3. Quality assessment

The risk-of-bias assessment was done by using the Cochrane Collaboration's tool. This assessment included 7 aspects: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other bias.

2.4. Zebrafish breeding

All zebrafish experiments were conducted at the Laboratory of Hunter Biotechnology, Inc., using AB strain zebrafish larvae. Larvae aged from 5 days post fertilization (dpf) to 6 dpf were selected, with inclusion criteria requiring full development of the swim bladder and the ability to swim actively. Fish were maintained in a recirculating aquatic system under standardized laboratory conditions, including a water temperature of 26–28 °C and a 14-hour light/10-hour dark photoperiod. The water composition was as follows: NaCl 3.5 g/L, $CaCl_2$ 0.1 g/L, KCl 0.05 g/L, and $NaHCO_3$ 0.025 g/L. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Hangzhou Hunter Biotechnology.

2.5. Maximum tolerated concentration (MTC)

Wild-type AB zebrafish larvae at 5 days post-fertilization (5 dpf) were randomly distributed into six-well plates, with 30 larvae placed in each well containing 3 mL of solution. Three types of groups were established: a normal control, a model control group, and five CBD dose groups. Except for the normal control, all groups were exposed to 5 μg/mL reserpine to induce a depressive model. CBD was administered as a water-soluble series of two-fold dilutions at concentrations of 5.00, 2.50, 1.25, 0.625, and 0.312 μg/mL. Larvae were maintained at 28 °C for 24 hours under static conditions without feeding. At the end of the exposure, the number of dead larvae in each well was recorded to calculate mortality, and the external morphology and behavioral status were compared with those of the model control. The maximum tolerable concentration (MTC) was defined as the highest CBD concentration that caused no mortality and produced no apparent abnormalities relative to the model control.

2.6. Chemical reagents and experimental design

Reserpine (purity ≥98%) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. A total of 5 μg reserpine was weighed and dissolved in 1 mL DMSO to prepare a 5 μg/mL stock solution. The concentration of reserpine was selected from previous studies on stress behavior in zebrafish (Chin, 2019). CBD (purity ≥ 99%) was obtained from Shanghai Yuansi Standard Science and Technology Co., Ltd. In the following experiment, larvae were divided into four groups: the normal control was exposed to standard zebrafish water as the control; the model control was exposed to 5 μg/mL reserpine for 24 hours; the positive control group was exposed to 5 μg/mL reserpine and 0.1 μg/mL of Sertraline hydrochloride for 24 hours; the CBD group was exposed to 5 μg/mL reserpine and 0.078 μg/mL, 0.156 μg/mL, and 0.312 μg/mL CBD respectively for 24 hours (Fig. 1).

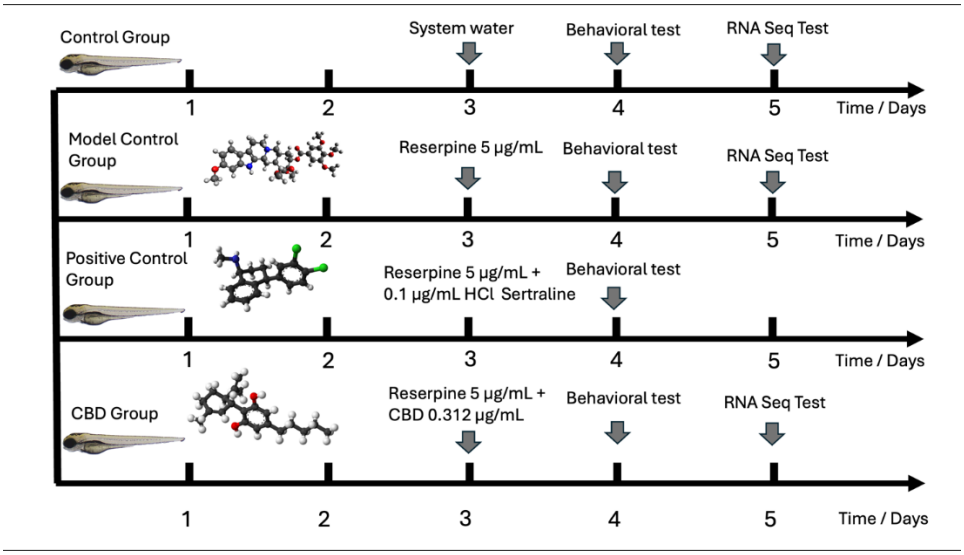

Fig. 1 Timeline of zebrafish cultivation

2.7. Behavior tests

After drug exposure, zebrafish larvae were transferred to a 24-well plate, with one larva in each well (Fig. 2). Each well had an inner diameter of 18 mm and contained 900 µL of zebrafish water. Using a 100 µL pipette, individual larvae were transferred from the corresponding group's 6-well plate. The plate was placed into a behavioral recording system (Zebra Lab 3.22.3.31, Viewpoint, France) The low detection threshold is set to 40. An infrared camera was used to automatically record the movement of each larva. During the test, larvae were first exposed to darkness for 5 minutes, followed by 20 minutes of light exposure. Larval activity was continuously tracked and recorded throughout the test.

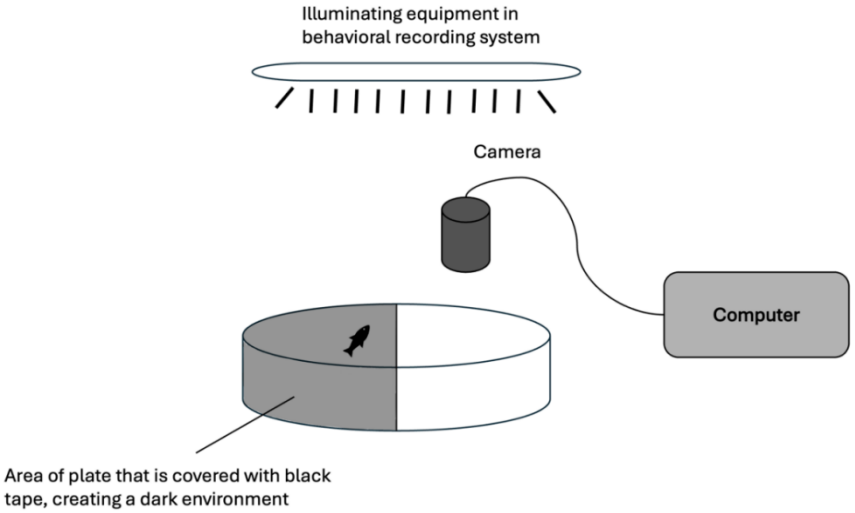

Fig. 2 Behavioral test - Light and dark box test

The dark section signifies the absence of light, creating a dark environment for the zebrafish. The bright section provides a normal lighting environment for the zebrafish. This is achieved using a 24-well plate.

2.8. RNA-seq

RNA-seq was done one day after the light-dark box test. All 90 samples of full fish were kept at the temperature of -80 ºC before further treatment. Milling beads and 700 µL of TRIzol lysis buffer (SKU 15596026, Invitrogen, USA) were added to each microcentrifuge tube before they were ground by a bead mill at a frequency of 60 Hz. The samples were then centrifuged for 3 minutes at a

temperature of 3 ºC and the centrifugal speed of 12000 G. After that, transfer 600 μL of supernatant from each microcentrifuge tube to the kit designed for automated RNA extraction. The extractor operated for 38 minutes to produce RNA samples, which were then centrifuged again for 5 minutes at a temperature of 4 ºC and the centrifugal speed of 12000 G. 1.5 μL supernatant from each sample was transferred to the probe of the nucleic acid and protein analyzer, Nanodrop 2000, through which RNA quality control was performed. After the completion of quality assessment, all the RNA samples were submitted to the BGI Genomics, Co., Ltd for RNA-seq.

The resulting file showed the expression level of all the genes in zebra fish from normal control, model control, and CBD groups, measured by several metrics, such as read count and fpkm. The read count data was taken out for two sets of comparisons: normal control vs model control and CBD vs model control. Both groups were processed using Science and Research online plot (SRplot, https://bioinformatics.com.cn/en) to perform DESeq2 differential gene expression analysis from the raw count matrix and gene set enrichment analysis, specifically gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Diagrams demonstrating the results were also plotted with SRplot. The DEG thresholds were $\log_2$fold change ≥ 1 or ≤ -1 and $p \leq 0.05$.

2.9. Statistical analysis

All statistical analyses were performed using SPSS software (version 26.0, IBM Corp., Armonk, NY, USA). Graphing was performed using GraphPad Prism, version 9.0 (GraphPad Software, San Diego, CA, USA). Data from each experimental group (N = 6 per group) were expressed as mean ± standard deviation. Group differences were first assessed using one-way analysis of variance (ANOVA). When significant effects were detected, Tukey's post hoc test was conducted to determine pairwise differences between groups. P-value ≤ 0.05 was considered statistically significant, indicating that the observed differences were unlikely due to chance.

3. Result

3.1. Literature search results

Total 78 studies were retrieved from the PubMed database, of which 54 articles were excluded by screening titles and abstracts. Among the 24 remaining articles for full-text assessment, 10 were excluded, and 14 studies were included in this study (Figure 3).

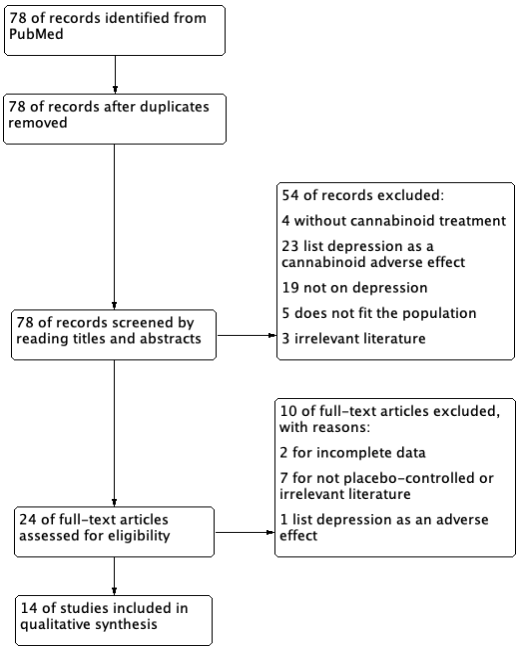

Fig. 3 Flow diagram of the literature screening process

Tab. 1 Basic characteristics of included articles

1st author (year)	Disease	Interventions	Outcomes
Alizadeh, 2022	Food addiction	Beta-caryophyllene (n=26), placebo (n=26)	DASS-21, no significance
Chaves, 2020	Fibromyalgia	THC-rich cannabis oil (n=9), placebo (n=9)	FIQ-Depression, no significance
Gundugurti, 2024	Mild to moderate anxiety	Nanodispersible CBD oral solution (n=89), placebo (n=89)	PHQ-9, significance
Hardy, 2022	Advanced cancer	CBD oil (n=70), placebo (n=72)	ESAS, no significance
Malik, 2016	Functional chest pain	Dronabinol (Δ9-THC) (n=10), placebo (n=9)	BDI, no significance
Mitarnun, 2025	Parkinson's disease	CBD (n=30), placebo (n=30)	HADS, no significance
Mosley, 2023	Tourette Syndrome	Placebo, then THC and CBD (n=11); THC and CBD, then placebo (n=11)	MADRS, no significance
Müler-Vahl, 2023	Chronic tic disorders	Nabiximols (Δ9-THC and CBD) (n=65), placebo (n=33)	BDI, no significance
Notcutt, 2004	Chronic pain	THC-rich cannabis oil, CBD-rich cannabis oil, THC: CBD (1:1) cannabis oil Sublingual spray administration	BDI and GHQ-28, no significance
Pinto, 2023	Acute bipolar depression	CBD (n=19), placebo (n=16)	PHQ-9, MADRS & HAMD, no significance
Raft, 1977	Dental Extraction	THC (n=3), Placebo(n=6)	ZDS and BDI, no significance
Toth, 2012	Diabetic peripheral neuropathic pain	Nabilone (Δ9-THC) (n=13), placebo (n=13)	HADS, no significance
Vela, 2021	Hand osteoarthritis and psoriatic arthritis	CBD (n=70), placebo (n=66)	HADS, no significance
Weber, 2010	ALS with cramps	THC then placebo (n=14), placebo then THC (n=13)	HADS, no significance

*BDI: Beck Depression inventory; *HADS: Hospital Anxiety and Depression Scale; *MADRS: Montgomery-Asberg Depression Rating Scale; *PHQ-9: Patient Health Questionnaie-9; *ZDS: Zung Depression Scale; *GHQ-28: General Health questionnaire-28; *HAMD: Hamilton Depression Rating Scale

3.2. Study characteristics

Table 1 provides a comprehensive synthesis of 14 placebo-controlled clinical trials evaluating the efficacy of various exocannabinoid interventions, including THC, CBD, beta-caryophyllene, and combination therapies. Exocannabinoid interventions using CBD (100-600 mg/day) and low-dose THC (1-5 mg/day) showed potential for treating anxiety, PTSD, and psychosis, although evidence remained limited. CBD appeared safer for psychiatric use, while THC required caution due to risks of anxiety and psychosis exacerbation. Balanced THC: CBD formulations may mitigate risks.

Key considerations included CBD's liver enzyme effects and drug interactions via CYP450 inhibition, THC's cognitive and cardiovascular side effects, and the need for close monitoring, particularly in schizophrenia or bipolar disorder. These studies encompassed diverse patient populations, including those with psychiatric disorders such as bipolar depression and anxiety; neurological conditions such as Parkinson's disease and Tourette syndrome; and chronic pain syndromes such as fibromyalgia and osteoarthritis. Sample sizes ranged from small-scale trials (9-16 participants per group) to larger studies (65-89 per group).

A key observation across these trials was that in most studies, depressive symptoms were evaluated as secondary outcomes associated with the primary conditions rather than as primary therapeutic targets. Consequently, the results demonstrated limited antidepressant efficacy, with 13 of 14 trials reporting non-significant outcomes on standardized psychological measures (BDI, HADS, MADRS, PHQ-9). The single exception was the study by Gundugurti et al. (2024), which reported significant improvement in depressive symptoms using a nanodispersible CBD oral formulation in patients with anxiety disorders.

Across the 14 trials, interventions varied widely in composition, dosing, and administration routes. CBD was typically administered at doses ranging from 100–600 mg/day (e.g., Gundugurti et al., 2024; Hardy et al., 2022), while THC doses were generally low (1–5 mg/day; e.g., Chaves et al., 2020; Mosley et al., 2023), with some studies using balanced THC: CBD formulations (e.g., Müler-Vahl et al., 2023). Notably, the sole study reporting significant antidepressant effects (Gundugurti et al., 2024)

employed a nanodispersible CBD formulation, suggesting enhanced bioavailability may be critical for efficacy. In contrast, trials targeting chronic pain (Notcutt et al., 2004), neurological disorders (Mitarnun et al., 2025), or comorbid conditions (Pinto et al., 2023) used heterogeneous measures (BDI, HADS, etc.) but consistently found no antidepressant benefits, possibly due to inadequate dosing, patient heterogeneity, or assessment of depression as a secondary outcome. Methodological limitations—such as small sample sizes (e.g., Raft et al., 1977; n=9), unclear dosing (e.g., Alizadeh et al., 2022), or crossover designs (Weber et al., 2010)—further complicate generalizability.

Overall, these findings highlight that while exocannabinoids show sporadic promise, their antidepressant potential likely depends on precise targeting (e.g., anxiety-related depression), optimized formulations, and trials specifically designed to assess depression as the primary outcome. The positive result with nanodispersible CBD in anxiety-related depression underscores the need for future research using standardized outcome measures and optimized dosing strategies in clinically depressed populations.

3.3. Summary of risk of bias assessment

Fig. 4A presents a summary of the risk of bias judgments for individual studies across seven domains, including selection bias, performance bias, detection bias, attrition bias, reporting bias, and other sources of bias. Each study was assessed using a color-coded system: green indicates low risk of bias, yellow denotes unclear risk, and red signifies high risk. Most studies showed a low risk of bias across the majority of domains, particularly in random sequence generation and allocation concealment. However, several studies displayed unclear risk in the domains of blinding of participants and personnel and blinding of outcome assessment. Notably, a few studies (e.g., Anaesthetist 2004, Hardy 2022, and Malik 2017) exhibit high risk in the "other bias" category, which may impact the overall reliability of their findings.

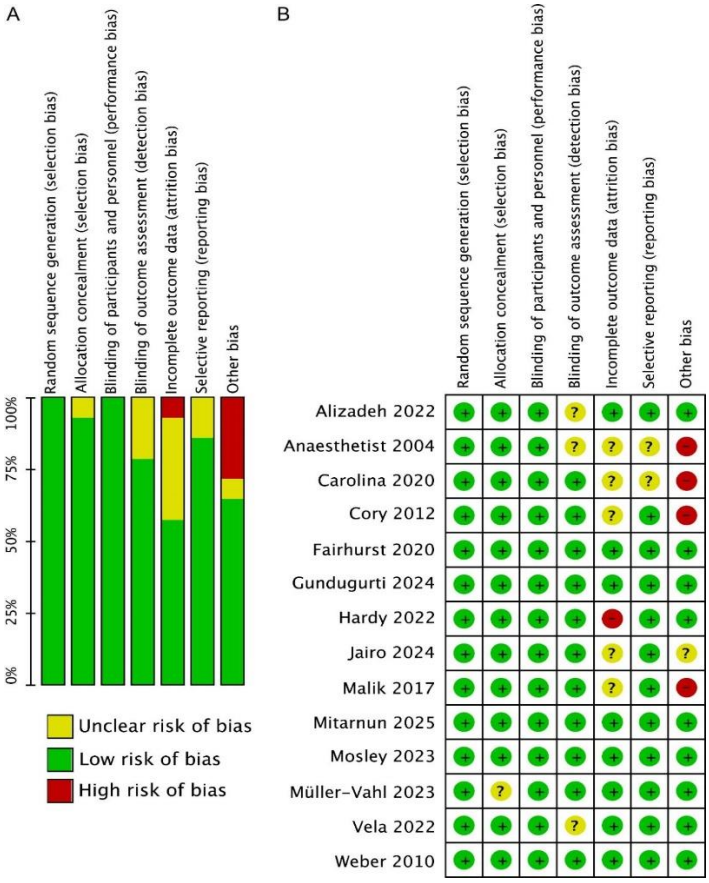

Fig. 4 (A) Risk-of-bias summary (B) Risk-of-bias graph

Fig. 4B provided a graphical overview of the proportion of studies assessed as having low, unclear, or high risk of bias in each domain. The bar chart confirmed that the majority of studies were judged

to have a low risk of bias in most domains. However, blinding of participants and personnel and blinding of outcome assessment show higher levels of uncertainty, with a substantial proportion rated as unclear. The domain with the most concern was incomplete outcome data, where a non-negligible proportion of studies were rated as high risk. Furthermore, the "other bias" domain demonstrated the highest proportion of high-risk ratings, indicating potential methodological concerns not captured in the standard domains.

Together, these figures highlighted both the strengths and limitations of the included studies. While most demonstrate methodological rigor in randomization and allocation, issues remained in blinding procedures and completeness of outcome data, which should be considered when interpreting the overall findings of the review.

3.4. CBD concentration affected the survival rate of zebrafish

Tab. 2 Concentration-dependent effects of CBD on zebrafish survival

Group	Concentration (μg/mL)	Number of deaths (fish)	Mortality rate (%)	Phenotypic observation
Normal control	-	0	0	No obvious abnormalities
Model control	-	0	0	No obvious abnormalities
CBD	0.312	0	0	Comparable to model control group
	0.625	24	80	-
	1.25	30	100	-
	2.50	30	100	-
	5.00	30	100	-

When the CBD concentrations were between 5.00 and 1.25 μg/mL, no zebrafish could survive. As the concentration decreased to 0.625 μg/mL, the mortality rate lowered to 80%. At 0.312, the mortality rate of zebrafish was exactly 0%, the same value as model control and normal control. Besides, from the phenotypic observation, most zebrafish at the 0.312 μg/mL concentration exhibited behavioral patterns similar to the normal control, with no obvious abnormalities, suggesting that this CBD concentration did not have a significant health impact on zebrafish. Hence, we concluded the maximum working concentration of CBD to be 0.312 μg/mL.

3.5. High-concentration CBD partially rescued the depressive behaviors of zebrafish models in light-dark box test

In order to assess the efficacy of CBD on depression, a light-dark box test was performed. The antidepressant effects of cannabidiol (CBD) in larval zebrafish were measured by the percentage of time spent and total distance traveled in the light environment (Fig. 5A). Fig. 5A epitomized the swimming trajectory of zebrafish from normal control, model control, positive control, and CBD groups (high-concentration, medium-concentration, and low-concentration) in light, respectively. A high density of trajectories shared by normal control, positive control, and high-concentration CBD group indicates a comparable phenotype with little observable abnormalities, while the low density of model control, medium-concentration, and low-concentration CBD groups suggests a depressive phenotype.

The untreated, wild-type larvae spend approximately 60% of their time in light, leaving a dense trajectory in the light field (Fig. 5B). Compared to the normal control group, chronic exposure to reserpine for 12 hours resulted in a significant reduction in time spent in light, indicating depressive-like behavior. Treatment with sertraline effectively reversed these effects, restoring time in light to levels comparable with the control group, thereby confirming its therapeutic efficacy against reserpine-induced depression. Likewise, high-concentration CBD significantly increased the percentage of time spent in light, showing no notable difference from the control group. In contrast,

low- and medium-concentration CBD did not produce significant improvements in time, suggesting lower efficacy than sertraline. The pattern of influence was mostly similar to that on distance travelled in light, except that high-concentration CBD only increased the distance moderately without a significant difference from the sertraline group (Fig. 5C).

Altogether, these findings suggest that high-concentration CBD (approximately 0.312 μg/mL) may exert partial antidepressant effects in this zebrafish model by increasing the percentage of time spent and total distance traveled in a light zone. Fig. 5B shows the percentage of time spent in the light. The model control group (M) spent significantly less time in the light than the normal control (NC), indicating increased anxiety. This behavior was improved in the positive control (PC) and treatment groups. Figure 5C shows the distance traveled in the light. Similarly, the M group moved a much shorter distance than the NC group. The PC and treatment groups showed a recovery in the distance traveled, suggesting the treatment reduced anxiety-like behavior.

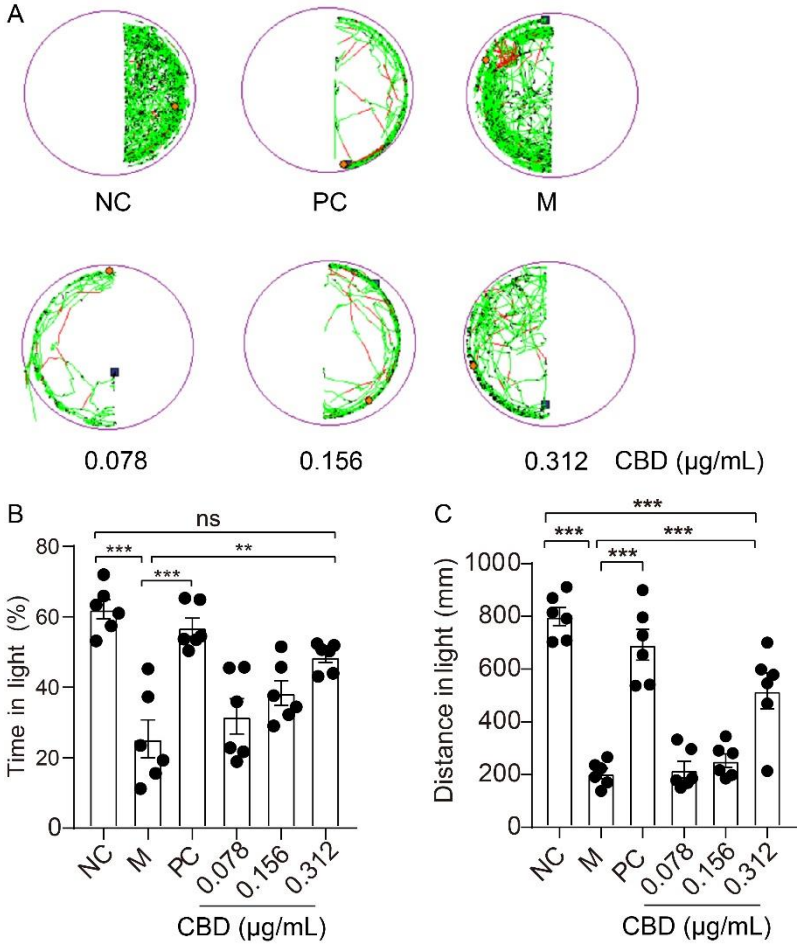

Fig. 4 Locomotor activity profiles of larval zebrafish exposed to sertraline and different concentrations of CBD after reserpine treatment in the light-dark box test. (A) Diagram of the locomotor activity recorded by a camera from a dorsal view. Histogram revealing the locomotion behavior in the light environment of larvae by the (B) time spent and (C) total distance traveled. NC, normal control; PC, positive control; M, model control. The data are expressed as the mean ± S.E.M. and were analyzed by one-way ANOVA followed by the Tukey post hoc test. Significance was defined as $^{**}p < 0.01$ and $^{***}p < 0.001$. ns, no significant.

3.6. Comparison of gene expression between the CBD and model control groups reveals activation of defense mechanisms

This volcano plot visualizes the differential expression analysis for the comparison of CBD treatment versus model control (M) (Fig. 6). The x-axis represents the $\log_2$ fold change, indicating the magnitude of gene expression differences, while the y-axis shows the $-\log_{10}$ p-value, reflecting

the statistical significance of these changes. The plot highlights 58 significantly up-regulated genes and 23 significantly down-regulated genes, based on pre-defined thresholds for fold change and p-value. Overall, the volcano plot effectively summarizes the minimal but specific transcriptional changes induced by CBD treatment, which is compared to the model control.

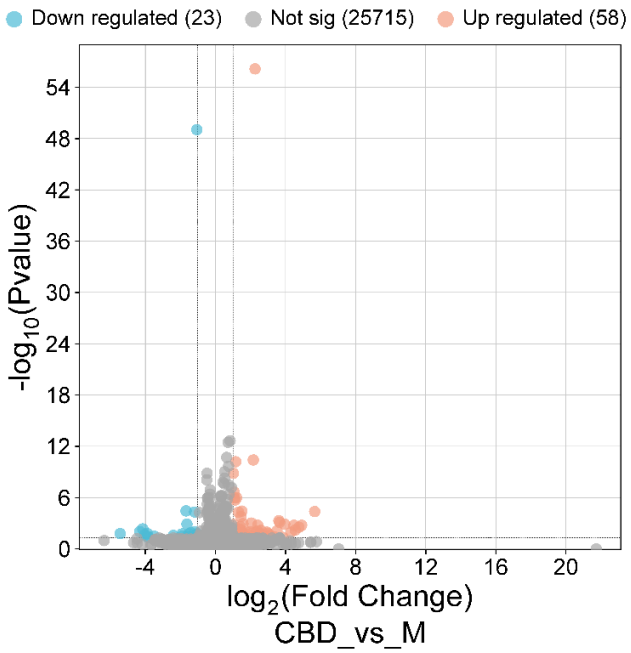

Fig. 5 Volcano plot shows gene expression changes in CBD vs M group.

Grey points: non-significant genes (n = 25,715). Blue points: significantly down-regulated genes (n = 23). Red points: significantly up-regulated genes (n = 58). X-axis shows $\log_2$ fold change (CBD/M); Y-axis shows statistical significance ($-\log_{10}$(p-value)). Dashed lines indicate significance and fold change thresholds.

The enrichment analysis reveals significant activation of biological processes related to "response to other organism," "response to external biotic stimulus," and "response to biotic stimulus" (Fig. 7A). Key defense-related processes—including "defense response," "defense response to other organism," "defense response to virus," and "defense response to symbiont"—show notably high enrichment scores, with $-\log_{10}$(p-value) values ranging from 3 to 8, indicating strong statistical significance. Additionally, "response to virus" and "xenobiotic catabolic process" are also significantly enriched. Overall, the plot highlights key processes involved in host-pathogen or symbiotic interactions, emphasizing defense mechanisms and responses to external biotic stimuli.

The cellular component enrichment analysis showed the AMPA glutamate receptor complex was most significantly enriched, followed by lipoprotein particles and the protein-lipid complex. Color intensity corresponded to significance levels, with all terms containing only 1 gene (Fig. 7B).

CBD primarily exerts its biological effects by modulating oxidoreductase and transferase activities in relation to defense, which ranked as the best enriched molecular functions (Fig. 7C). Heme binding and monooxygenase activity followed, indicating that CBD treatment might moderately affect the cytochrome P450 enzyme system. UDP-glycosyltransferase activity was enriched at a similar level, suggesting the potential involvement of CBD in xenobiotic metabolism and detoxification processes. Smaller dots, such as chitin binding and laminin binding, were the least significant, possibly related to cellular structure maintenance.

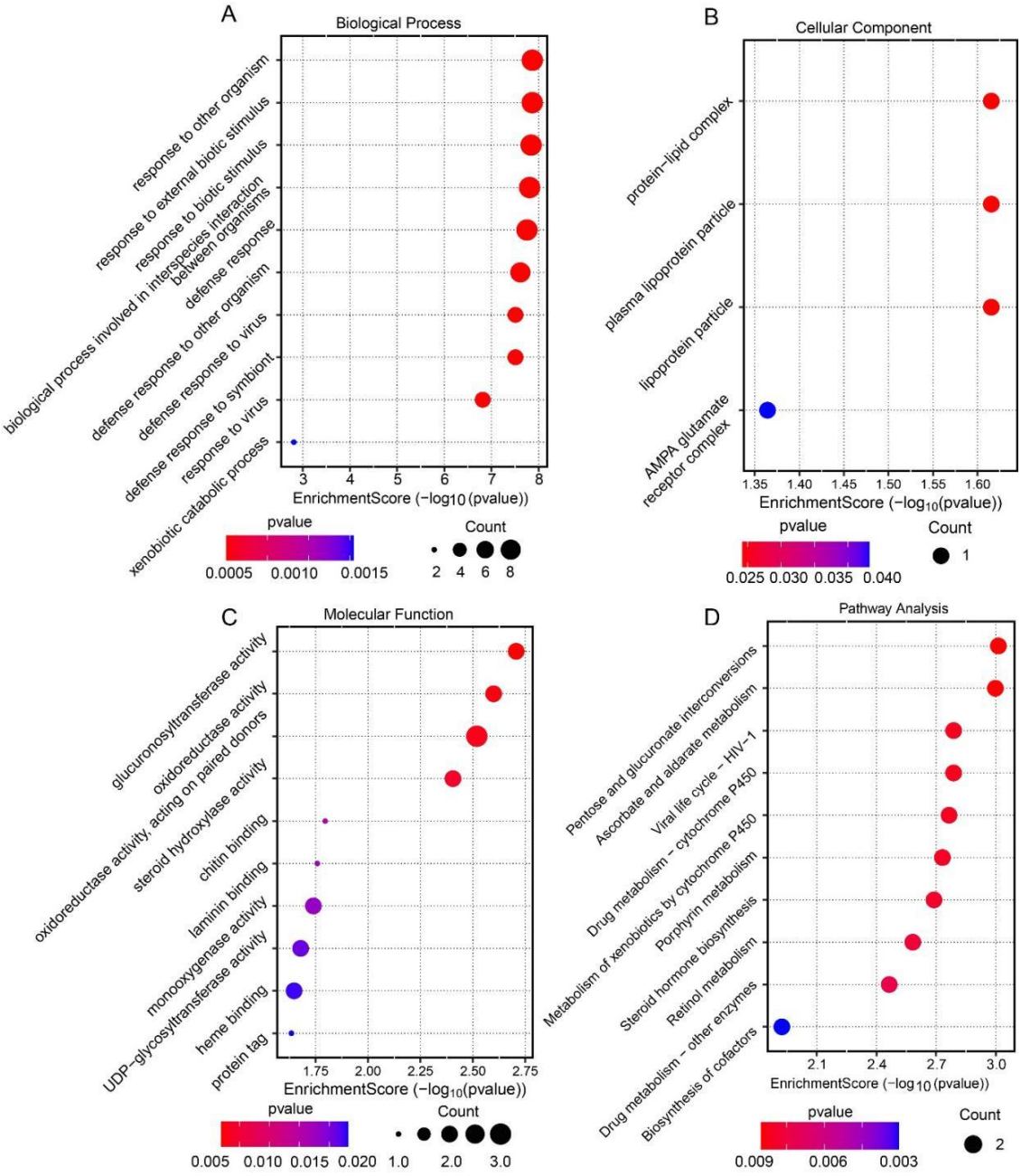

Fig. 6 GO and KEGG pathway enrichment among DEGs in CBD vs M.

X-axis: enrichment score (–log₁₀(p-value)); Y-axis: specific processes. Dot size indicates gene count; color shows p-value significance.

Pathway analysis showed steroid hormone biosynthesis and cytochrome P450 drug metabolism were most significant (p≈0.003), indicating CBD strongly affects hormone metabolism and detoxification (Fig. 7D). Retinol metabolism and xenobiotic metabolism were also highly enriched (p≈0.006), suggesting roles in vitamin metabolism and detoxification. Pathways like pentose/glucuronate interconversions (p≈0.009) show CBD's effects on antioxidant metabolism. These demonstrate that CBD primarily modulates drug metabolism, hormone synthesis, and antioxidant defense through P450 system regulation.

3.7. CBD promoted neural recovery via synaptic and structural remodeling

To explore the effects of reserpine on the neurological function of the zebrafish model, we compared model group and control group. It indicates 327 significantly downregulated genes, 47 significantly upregulated genes (Fig. 8).

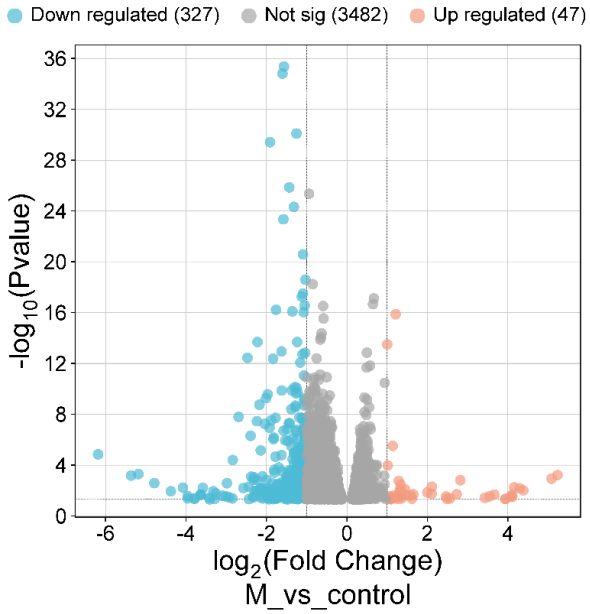

Fig. 7 Volcano plot visualizing the differential gene expression analysis between model group (M) and the control group.

Grey points: non-significant genes (n = 3482). Blue points: significantly down-regulated genes (n = 327). Red points: significantly up-regulated genes (n = 47). X-axis shows $\log_2$ fold change (M/Control); Y-axis shows statistical significance ($-\log_{10}$(p-value)). Dashed lines indicate significance and fold change thresholds.

The biological process analysis indicates significant enrichment in processes related to neuronal development and synaptic functions (Fig. 9A). Axon development shows the highest enrichment, where neuron projection morphogenesis and regulation of cytoskeleton organization are the most significant. Overall, this suggests that the genes play crucial roles in neuronal structural formation and synaptic signaling pathways.

Cellular component analysis showed the extracellular matrix and collagen-containing extracellular matrix were most significant with 90-110 genes, indicating CBD treatment profoundly affects extracellular microenvironment (Fig. 9B). Medium-colored focal adhesion and cell-substrate junction involving 50-70 genes suggest CBD modulates cell adhesion functions. Lighter points, including actin cytoskeleton and intermediate filaments, show CBD's regulation of cytoskeletal structure. These demonstrate that CBD primarily influences cellular structure and intercellular interactions by modulating extracellular matrix and cell junctions.

Molecular function analysis revealed that the darkest point, extracellular matrix structural constituent, was most significant with ~125 genes, indicating CBD treatment strongly affects extracellular matrix integrity (Fig. 9C). Medium-colored ionotropic glutamate receptor activity and ligand-gated ion channel activity were highly enriched with 75-100 genes, suggesting CBD modulates neural signaling. Lighter points, including actin binding, show CBD's regulation of cytoskeletal dynamics. These demonstrate that CBD primarily exerts effects by modulating extracellular matrix structure, ion channel function, and cytoskeletal binding activities.

Bar graph displaying enriched pathways including cytoskeleton regulation, cell adhesion, and signaling pathways (Fig. 9D). Pathway analysis showed ECM-receptor interaction and focal adhesion were most significant, indicating CBD strongly affects cell-matrix interactions. Regulation of actin cytoskeleton and cell adhesion molecules was highly enriched, suggesting CBD modulates cell motility and adhesion. The MAPK signaling pathway shows CBD's role in signal transduction. These demonstrate CBD functions through cell-matrix interactions, cytoskeletal reorganization, and signaling pathways.

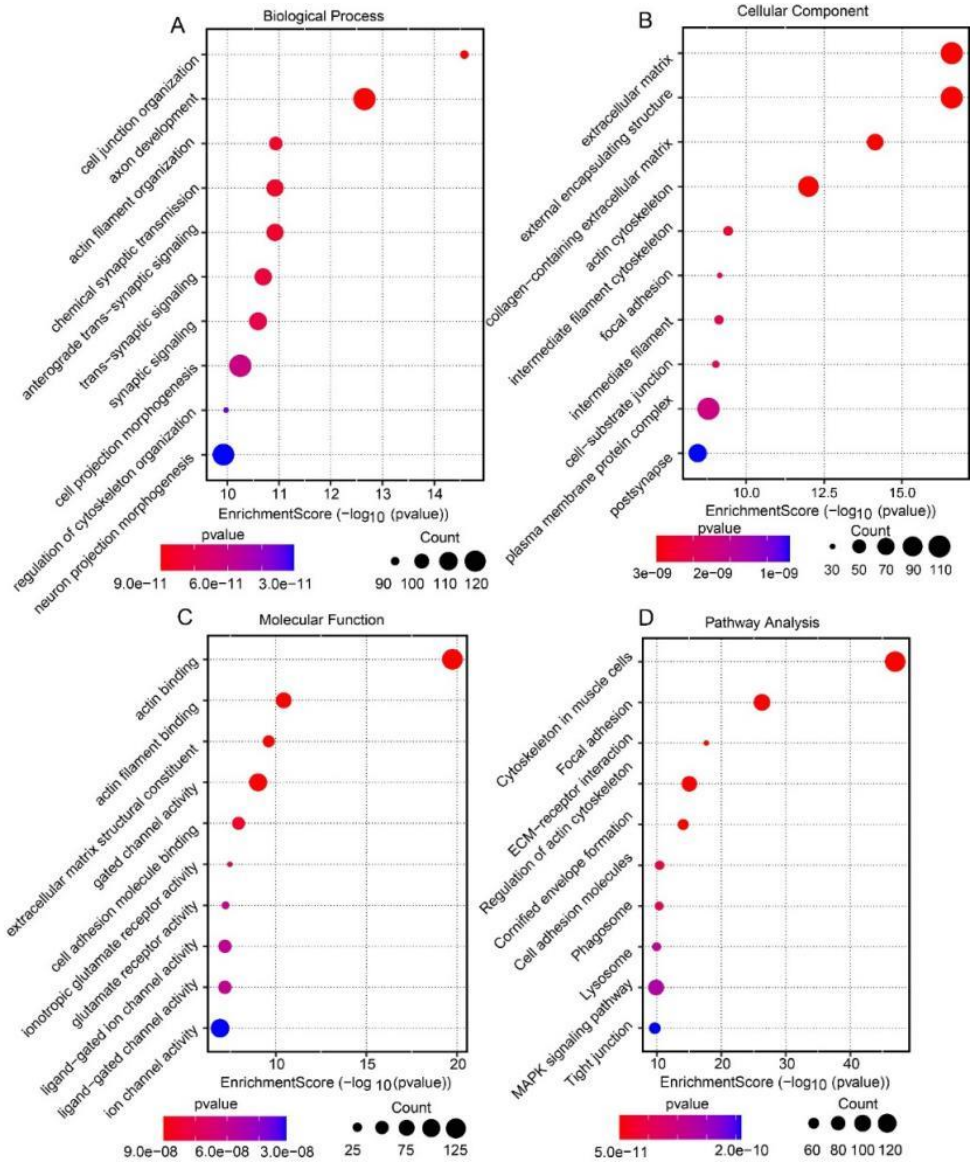

Fig. 8 GO and KEGG pathway enrichment among DEGs in M vs control.

X-axis: enrichment score (–log₁₀(p-value)); Y-axis: specific processes. Dot size indicates gene count; color shows p-value significance.

3.8. CBD may mitigate depressive phenotype of zebrafish model by upregulating the expression of interferon-stimulated genes

To explore which and how changes in the process of transcriptional regulation affected the reserpine-induced zebrafish model, we traced genes that experienced changes in expression level in both sets of comparisons (CBD vs model and model vs control), using a Venn diagram (Fig. 10). 19 genes in the intersection of the diagram revealed a core set of genes that were significantly dysregulated in the depression-like model and subsequently normalized to control levels by CBD treatment. From this shortlist, genes were further prioritized based on their involvement in biologically relevant pathways, as revealed by GO enrichment analysis (e.g., neuroimmune pathways), pinpointing key candidates like *IFIT15* and rsad2 for downstream experimental validation.

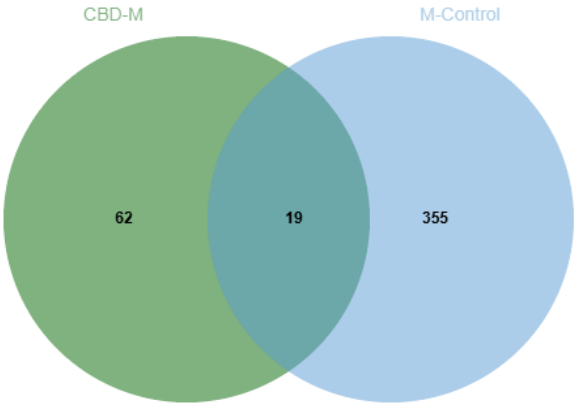

Fig. 9 Differentially expressed genes in both CBD vs M and M vs Control groups

Both Interferon-induced tetrapeptide repeat 15 (*IFIT15*) and radical SAM domain–containing protein 2 (*RSAD2*) are interferon-stimulated genes (ISGs) involved in immune responses (Helbig & Beard, 2014). The enrichment of these 2 genes in the immune response pathway echoes with an inflammatory hypothesis of depression. Interestingly, the expression level of both was found to be downregulated from control to model group and upregulated from model to CBD group to a higher level than it was in the control group. Hence, it is predicted that CBD may participate in the mentioned inflammatory response by stimulating the expression of the two ISGs after binding to certain receptors, which could potentially be CB1, and eventually inhibiting depressive phenotype (Fig. 11).

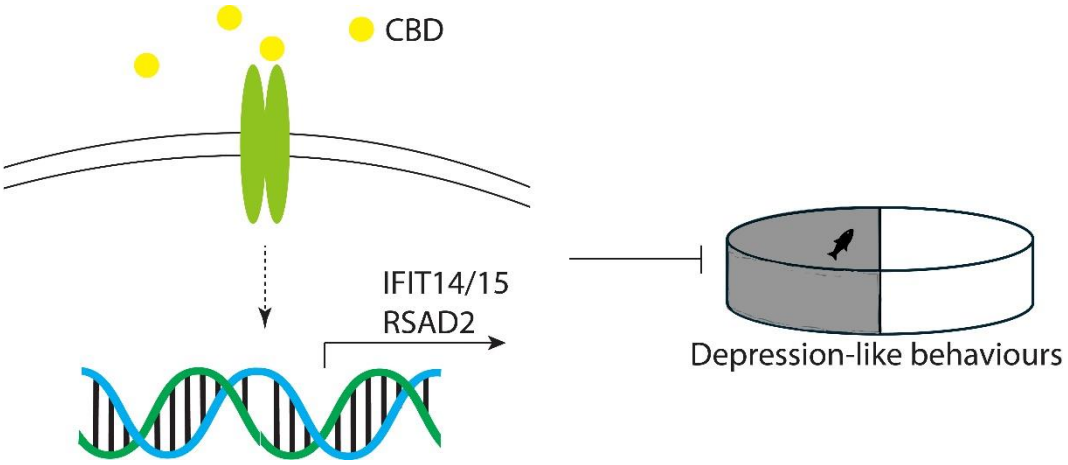

Fig. 10 Potential pathways whereby CBD modulates ISGs to alleviate depression

Besides *IFIT15* and *RSAD2*-mediated inflammatory response, other potential antidepressant mechanisms of CBD were also discovered, which are mainly centered around the reshaping of synapses and metabolism. The downregulated TMEM255B and SERPINE2, and the upregulated GLRA4a and SPTBN5, all function in synaptic transmission and synaptic plasticity. Notably, TMEM255B regulates the functions of NMDA receptors, which bind with glutamate, a major excitatory neurotransmitter in the central nervous system. TMEM255B may, therefore, mitigate depression by inhibiting glutamatergic excitotoxicity from model to CBD group. Meanwhile, metabolism is coordinated by the downregulated SLC5A1 and the upregulated NR0B2A, APOF, and RSAD2. While RSAD2 can ameliorate inflammation, it also limits oxidative damage and improves cellular respiration. This second function could effectively counteract depressive pathology resulting from massive neuron death caused by mitochondrial ROS accumulation.

4. Discussion

Based on previous RNA-seq results and analysis, our research proposed inflammation as one of the most likely mechanisms underlying depression. Neuroinflammation has been proven to be involved in the pathophysiology of depression, featuring the activation of glial cells and the release of inflammatory cytokines (Sălcudean et al., 2025). Together, neuroinflammation and depression perpetrate a vicious cycle: not only can inflammation in the CNS contribute to the onset of depressive symptoms, but depression itself can also exacerbate inflammatory responses (Beurel et al., 2020). Their bidirectional relationship is also suggested by the therapeutic efficacy of antidepressants on neuroinflammation. For example, clinical trials shed light on the capacity of ketamine to reduce NIF levels. Correspondingly, depressive symptoms were correlated with reductions in levels of NIFs (e.g., IL-6 and IL-17A) (Zhan, 2020; Zhou, 2021). These findings altogether nominate neuroinflammation as both a pathogenic mechanism of depression and a promising therapeutic target. In our study, it is speculated that CBD treatment could, likewise, combat a systemic inflammation response, which might have been triggered by the process of modeling, thereby rescuing the depressive-like phenotype in the zebrafish model.

In our research, the depressive zebrafish model was induced by dissolving reserpine in the breeding environment, and full fish samples were taken to conduct RNA-seq. We infer that systemic inflammation contributed to the onset of depression, starting with a leak of peripheral immune cells and cytokines through the blood-brain barrier (BBB) (Wilson et al., 2010). They can then activate microglia and astrocytes. However, once pro-inflammatory microglia are excessively activated and start to release lavish amounts of cytokines, neurons become damaged by the neurotoxic cytokines (Wang et al., 2022; Beurel et al., 2020). As a result, neuroinflammation takes place. Moreover, peripheral cytokines may inhibit neuroplasticity and neurogenesis in the CNS, preventing the reparation of impaired neurons (Fernezelian et al., 2024). Neurotransmitters are also affected. For instance, glutamate reuptake is blocked, depositing excitotoxicity in neurons (Miller et al., 2009; Miller & Raison, 2015). Likewise, inflammatory cytokines can lower the activity of the vesicular monoamine transporter 2 (VMAT2) and the dopamine transporter (DAT), reducing dopamine levels (Miller & Raison, 2015). Zooming out to an organismal level, the HPA axis is immobilized from regulating the negative feedback loop. Consequently, the immune imbalance works in tandem with HPA axis dysfunction to co-construct yet another malicious cycle of depression and inflammation (Gerritsen et al., 2018).

Interferon-stimulated genes (ISGs) are typically upregulated upon interferon signaling and, under pathological or aging conditions, may drive excessive neuroinflammation and contribute to the development of inflammation-related diseases such as neurodegenerative disorders and post-stroke syndromes (Blank & Prinz, 2017). However, in our reserpine-induced depression model, we observed a downregulation of ISG expression, suggesting that reserpine treatment leads to insufficient immune activation rather than hyperactivation. This aligns with evidence that both insufficient and excessive microglial activation can contribute to depressive phenotypes (Yirmiya et al., 2015).

Importantly, ISGs play context-dependent roles influenced by developmental stage and pathological status: in aging or neurodegenerative contexts, ISG upregulation may act as pro-inflammatory mediators, while in younger, physiologically healthy states, ISGs might function as stabilizers of emotional homeostasis (Baruch et al., 2014). Given that our experiments were conducted in juvenile zebrafish, we interpret ISG upregulation in this setting as serving an emotional-stabilizing role.

Among ISGs, RSAD2 (viperin) has known immunomodulatory functions. Notably, Mecha et al. (2013) demonstrated in a viral model of multiple sclerosis (MS) that cannabidiol (CBD) increases IFN-dependent transcripts, including RSAD2, contributing to anti-proliferative activity in T cells. Our findings—where RSAD2 regulation in the depression model aligns with this direction—further reinforce the conclusion that ISG (including RSAD2) modulation is part of CBD's therapeutic mechanism.

Mechanistically, under normal physiological conditions, ISGs are tightly regulated to enable appropriate immune response initiation. In the reserpine model, suppressed ISG expression likely hindered this immune response activation. Following CBD treatment, ISG expression—including RSAD2 and related transcripts—was restored, suggesting that CBD promotes initiation of proinflammatory signaling necessary for proper neural function. Thus, CBD's antidepressant efficacy likely stems from targeted ISG induction, rebalancing immuno-neural homeostasis in developing brains.

Reconnecting to the ECS, a potential therapeutic target for depression that could enhance monoamine neurotransmitters and brain-derived neurotrophic factor (BDNF) levels, as well as increasing neurogenesis after injection of CB1 agonists (Poleszak et al., 2019; Zimmermann et al., 2018; Dong et al., 2019). Current studies hold controversial views towards its role in inflammation and immunity (Rahaman & Ganguly, 2021). Variations in cell type, receptor expression level, and ligand sensitivity among a multitude of immune cells may explain such discrepancies (Pandey et al., 2009). In the CNS, specifically, the effects of endocannabinoids are related to activation states of microglia. For example, when pro-inflammatory M1 types are activated, CB2 expression declines, whereas when anti-inflammatory M2a types are activated, CB2 expression increases (Mecha et al., 2016). Mecha et al. (2015) also reported that 2-Arachidonoylglycerol (2-AG) and Anandamide (AEA) could induce the expression of M2, or anti-inflammatory, phenotype biomarkers, highlighting the importance of endocannabinoids in reinforcing restorative microglia. Therefore, we propose that CBD activates M2a, thereby suppressing inflammation. Altogether, these findings suggest ECS as a promising target for depression.

Another reason for differences in results is the varied doses across studies. As shown in the meta-analysis, the therapeutic effects of CBD on depression were not generally significant. Within the limit of MTC, high concentrations might cause toxicity, inhibiting locomotor activity of zebrafish, similar to the behavioral effects brought by low concentrations (Licitra et al., 2022). This could be because varying CBD concentrations trigger different microglial phenotypes and thus result in different extents of inflammation (Mecha et al., 2015), as our results suggested.

Future studies should further clarify the role of microglia in ECS-mediated antidepressant effects. Since both insufficient and excessive microglial activation are linked to depressive phenotypes, dissecting how CBD modulates microglial states in specific brain regions will be critical. Brain-tissue–specific and cell-type–focused studies could validate whether the observed ISG upregulation indeed reflects protective immune activation rather than pro-inflammatory signaling.

In addition, individual variability may significantly affect the antidepressant efficacy of CBD. Clinical data suggest that differences in metabolic rates, genetic background, and comorbid conditions can alter both ECS regulation and drug safety. Therefore, future work should not only prioritize dose optimization but also incorporate personalized treatment strategies tailored to individual characteristics, ultimately improving both the safety and effectiveness of ECS-targeted interventions.

5. Conclusion

In conclusion, our study demonstrated that high-concentration CBD partially reversed depressive-like behaviors in a zebrafish model. Mechanistically, CBD restored immune balance by upregulating ISGs and modulating neuroimmune pathways. These findings highlight the ECS as a promising therapeutic target for depression and provide molecular insights for future drug development.

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