

Review Article

Cannabis-induced of behavioural effects in zebrafish: A comprehensive review

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ABSTRACT

The rising use of cannabis and its constituents for therapeutic and recreational purposes has intensified interest in understanding its neurobehavioral effects. Zebrafish (*Danio rerio*) have emerged as a robust model for behavioral neuroscience due to their genetic similarity to humans and conserved endocannabinoid system. This narrative review explores the behavioral effects of major cannabinoids including Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD) on anxiety, learning and memory, and social interaction in zebrafish. Findings indicate that THC induces dose-dependent anxiogenic effects, particularly at higher concentrations, characterized by hypolocomotion and increased bottom-dwelling. Conversely, CBD shows anxiolytic potential at moderate doses but may produce biphasic effects depending on dose and exposure timing. In cognitive assays, THC impairs learning and memory performance in spatial and associative tasks, while CBD's effects vary based on administration phase. Limited studies on social behavior suggest cannabinoids may modulate sociability, although outcomes remain inconsistent. This review highlights methodological gaps, such as the underrepresentation of chronic exposure studies, sex-based analyses, and minor cannabinoids like CBC, CBG, and CBN. Furthermore, few studies integrate behavioral outcomes with molecular or neurophysiological endpoints. By consolidating current findings, this review underscores the potential of zebrafish as a translational model in cannabinoid research and advocates for more standardized, mechanistically informed investigations to clarify the behavioral impact of cannabis constituents.

Keywords:

Behavior; Cannabidiol; Cannabinoids; Tetrahydrocannabinol; Zebrafish

1. INTRODUCTION

Cannabis sativa, a plant with a rich history of medicinal and recreation use is known to have over 100 phytocannabinoids in it with each exhibiting unique pharmacological profiles¹. Among them, there are THC which is a well-known psychoactive cannabinoid extracted from the plant due to its euphoric effects and potential therapeutic applications². CBD is another prominent cannabinoid found in the plant. It has garnered attention due its non-psychoactive properties, which

make it potential therapeutic treatment for variety of neurological disorders². Besides THC and CBD, there are also lesser-known cannabinoids such as cannabinol (CBN), cannabigerol (CBG) and cannabichromene (CBC) that are increasingly being studied for their distinct effects on the central nervous system and potential therapeutic benefits. Preliminary studies suggest that these compounds may influence behavioral outcomes in zebrafish, although comprehensive analyses are limited. Cannabinoids can interact with the body through the cannabinoid receptors in the the cannabinoid receptors

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in the endocannabinoid system (ECS) and produce many biological effects¹.

ECS, a complex cell-signalling system, is first identified in the early 1990s where it plays a crucial role in regulating many physiological processes including mood, memory, appetite and pain sensation. ECS comprises of endogenous cannabinoids (endocannabinoids), cannabinoid receptors and enzyme responsible for the synthesis and degradation of endocannabinoids³. There are many cannabinoid receptors in ECS, primarily cannabinoid receptor 1 (CB₁) and cannabinoid receptor 2 (CB₂) which are commonly known related to the interaction of cannabinoids in the body. CB₁ receptors are located predominantly in the central nervous system (CNS) that influence the neurotransmitter release, while CB₂ receptors are mainly found in peripheral tissues and function in modulating the immune responses. ECS can be found not only in human body but also other animals including others animal model such as zebrafish. which is widely used as animal model in current research of pharmacology³.

Zebrafish, commonly known as *Danio rerio*, is a popular animal model in modern neuroscience and pharmaceutical research. Zebrafish have emerged as a promising animal model due to their genetic resemblance to humans, transparent embryonic development, and capacity to perform high-throughput screening⁴. The fully sequenced zebrafish genome has shown that approximately 70% of human protein-coding genes have counterparts in zebrafish, and about 84% of genes associated with human diseases have homologous genes in the zebrafish genome⁵. Zebrafish also have a completely functional ECS, including CB₁ and CB₂ receptors that are distributed, and analogous to those in mammals. The expression of CB₁ receptors in zebrafish begins as early as 24 hours post-fertilization, with increased expression in brain structures observed by 48 hours post-fertilization⁶. The property allows for behavioural experiments to be performed on the animal model.

Various behavioural paradigms have been developed to assess anxiety, cognition, and social interaction in zebrafish (Figure 1). Behavioural assays in zebrafish include the novel tank diving test (NTDT), light and dark preference test and social interaction test which all have been effectively utilized to assess anxiety-like behaviour, locomotor activity and social behaviour⁴. The others assay such as active avoidance task and Pavlovian fear conditioning are used to assess learning and memory in zebrafish⁴. In this study, these assays have revealed that acute exposure to THC can induce concentration-dependent behavioral changes, including hypoactivity and altered stress responses. Similarly, CBD has been shown to modulate these effects, suggesting a complex interplay between

different cannabinoids. These behaviour observations provide insights into the neurobehavioral effects of various compounds including cannabinoids.

This review aims to provide a comprehensive overview on the behavioural effects of the cannabis and its constituents tested in zebrafish models. The review will be discussed on the pharmacodynamics of major cannabinoids and summarise the behavioural assays employed in zebrafish model. By looking from the previous studies, the review will highlight the translational relevance for zebrafish to be used in cannabinoid research and identify the potential gaps for future investigations.

Understanding the behavioural effects of cannabinoids is needed with given to the increase in legalization and medicinal use of cannabis worldwide. Zebrafish as animal model can offer a cost-effective and favourable alternative for the long-used others animal models which allow for rapid screening of cannabinoids effects on behaviour. The insights gained from zebrafish studies can be used to inform clinical research, aid in the development of cannabinoid-based therapeutics and contribute to public health regarding cannabis use.

1.1. Behavioral studies assessing anxiety in zebrafish

Anxiety is characterized by heightened arousal and behavioral inhibition in response to perceived threats or novel stimuli. In zebrafish, anxiety-like behaviour is commonly assessed using paradigms that exploit their natural aversions and exploratory tendencies⁷. The anxiety-like behavior of zebrafish was evaluated using NTDT and light–dark preference test, as illustrated in Figure 1(A) and Figure 1(B). NTDT where anxious fish tend to remain at the bottom of a new tank, and the light-dark preference test, where the increase in time spent in the dark zone reflects anxiety-like avoidance behaviour⁷⁻⁸. Other tests, such as the Open Field Test (OFT) and the Visual Motor Response (VMR) assay use measurements like distance travelled, freezing behaviour, and velocity to infer anxiety states⁹⁻¹⁰. These behavioural assays are not only non-invasive and high throughput but are also sensitive to pharmacological manipulations which include cannabinoid exposure. These support that the fact zebrafish provides reliable and translationally relevant model for evaluating anxiogenic or anxiolytic potential of cannabis-derived compounds such as THC and CBD.

1.2. Behavioral studies assessing learning and memory in zebrafish

Learning and memory involve the processes of acquiring, storing and retrieving information in response

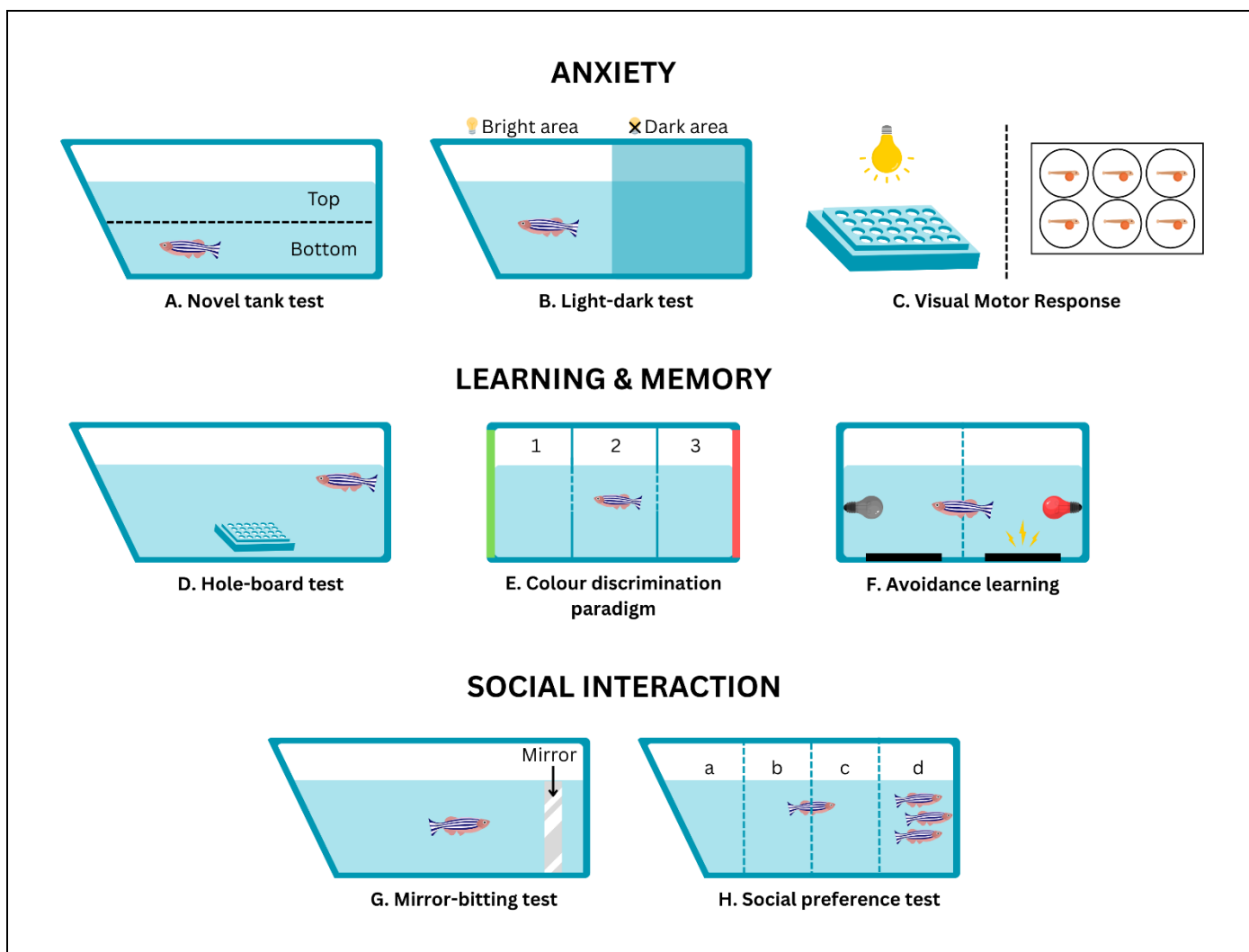


Figure 1. Behavioral assays commonly used to assess anxiety, learning and memory, and social interaction in cannabis induced zebrafish.

to environmental stimuli. In animal models, these cognitive functions are typically taken from behavioural adaptations to repeated or conditioned experiences. For zebrafish, learning and memory are commonly assessed using behavioural assays that involve conditioning or reward-based tasks⁴. The active avoidance task is frequently used to measure associative learning, where zebrafish learn to avoid a compartment associated with a mild aversive stimulus⁴. The Pavlovian fear conditioning paradigm evaluates memory formation by associating visual or environmental cues with discomfort that often leading to increasing freezing or bottom-dwelling as indicators of fear memory⁴. Other paradigms such as the hole-board spatial cognition test (Figure 1(D)), assess spatial learning by training zebrafish to locate a baited area in an open environment¹¹. Additional methods including colour discrimination tasks and two-alternative choice tests, have been utilized to examine decision-making, memory retention, and reversal learning¹¹. These tasks are sensitive to disruptions caused by cannabinoids such as THC and CBD, which have been shown to alter cognitive performance in a

dose and timing dependent manner. Collectively, these assays reinforce the utility of a zebrafish as a translationally relevant model for investigating cannabinoid-induced cognitive effects.

1.3. Behavioral studies assessing social interaction in zebrafish

Social interaction is a fundamental behavioural domain often used to evaluate sociability, group cohesion, and social preference in animal models. In zebrafish, social behaviours such as shoaling, mirror interaction, and preference for conspecifics are well-characterized and have become important indicators in neurobehavioral studies⁴. Social behavior was evaluated using the mirror-biting test and social preference test (Figure 1(G)) and Figure 1(H)), which are commonly used to assess aggression and sociability in zebrafish. Zebrafish are inherently social animals, making them highly suitable for investigating alterations in social functioning due to pharmacological or environmental interventions¹². Several behavioural

assays have been developed to assess social interaction in zebrafish. The social preference test measures the time spent near a compartment containing conspecifics compared to an empty area, with increased proximity indicating normal or enhanced sociability⁴. Another widely used assay is a mirror biting test, which evaluates aggressive or social responses based on the fish's reaction to its own reflection⁴. Time spent near the mirror along with other social behaviour activities such as biting, charging or colour changes provides insights into the fish's social reactivity¹³. Nevertheless, the zebrafish model remains a practical and translational tool for investigating cannabinoid-related changes in social functioning.

2. DISCUSSION

The studies compiled in the matrix table of Table 1, Table 2, and Table 3 which were selected primarily from publications within the past five years, with some extending up to 11 years. This selection offers a broad overview of the behavioral effects of cannabis in zebrafish models, highlighting how cannabinoids such as THC and CBD influence behavior across different developmental stages and experimental contexts.

The behavioral evidence across anxiety, learning and memory, and social interaction domains in zebrafish models highlights both the utility and complexity of this model in cannabinoid research. In anxiety-related assays (Table 1), findings show that both THC and CBD exert dose-dependent and developmental-stage-specific effects. THC commonly induces anxiogenic responses, particularly at higher concentrations, as seen in increased bottom-dwelling, reduced swimming velocity, and prolonged immobility in adult zebrafish^{7,16}. However, early-life exposure results were mixed where at low doses showed minimal behavioral changes, higher doses were lethal before adulthood⁹. This suggests a biphasic response, where low-dose exposure elicited minimal behavioral effects, while higher doses resulted in developmental toxicity and lethality. In contrast, CBD generally exhibited anxiolytic effects, particularly at mid-range doses though this response was nonlinear¹³. At both low and high doses, the effect diminished or became statistically insignificant, suggesting a narrow therapeutic window. For instance, CBD has been reported to exhibit non-linear dose-response effects. In one study, both low (0.5 µg/mL) and high (10 µg/mL) concentrations reduced locomotor activity in zebrafish larvae, indicating a lack of dose-dependent inhibition. However, the higher concentration (10 µg/mL) uniquely attenuated behavioral responses to darkness, suggesting that CBD may exert distinct modulatory effects at different exposure levels rather than a simple linear dose-response relationship¹⁴.

In learning and memory assessments (Table 2), THC exposure consistently impaired performance across various cognitive tasks. For instance, acute THC administration significantly increased latency and swim distance in spatial learning tasks, indicating memory retrieval deficits¹¹. Chronic THC exposure also disrupted avoidance learning, with fish failing to meet learning criteria¹⁸. CBD's impact was more complex; pre-training administration at low doses preserved memory, while post-training exposure at the same dose impaired retention¹³. These findings imply that cannabinoid effects on cognition are not only dose-dependent but also sensitive to the timing of administration relative to learning phases. However, studies rarely incorporated neurobiological data to validate behavioral findings, limiting their explanatory power.

Social interaction studies are comparatively scarce and yield inconsistent results (Table 3). A single-dose CBD injection did not significantly affect aggression or sociability in zebrafish¹³; whereas exposure to CBD- and THC-rich oils increased time spent with conspecifics, suggesting enhanced sociability.²⁰ These contrasting findings may reflect differences in formulation, dosage, or test sensitivity. Notably, minor cannabinoids like CBC, CBG, and CBN remain unexplored in this behavioral domain, despite their growing pharmacological relevance.

Methodologically, the reviewed studies differ considerably in exposure protocols, behavioral assays, and developmental stages assessed, contributing to the observed heterogeneity. While many studies rely on acute exposure models, the absence of chronic or longitudinal designs leaves open questions about the lasting impact of cannabinoids on zebrafish behavior. There is also a lack of sex-based analysis, standardization of behavioral endpoints, and integration of molecular markers. Addressing these limitations is critical for enhancing the translational value of zebrafish in cannabinoid research and producing more conclusive insights into cannabis-induced behavioral effects.

3. STUDY LIMITATION

Despite the expanding literature on cannabinoid-induced behavioural effects in zebrafish, several limitations remain apparent across existing studies. A major issue is the lack of standardized methodologies regarding cannabinoid dosage, exposure timing, developmental stages, and behavioural assay protocols, which complicates cross-study comparisons. While most studies have focused on THC and CBD, research on minor cannabinoids such as CBG, CBN, and CBC is still limited, leaving their behavioural effects largely unexplored. Additionally, the majority

Table 1. Anxiety behavioral assay for cannabis-induced zebrafish.

Cannabis Treatment	Zebrafish Stage	Behavioural Assay	Test Duration	Parameters	Findings	Ref.
THC: 1.0 mg/mL, 30 min, exposure	Adult AB/wild type (3 months)	Novel Tank Diving Test	10 min	Time spent in bottom zone Swimming velocity (cm/s) Time spent immobile (s)	THC exposure increased bottom-dwelling, reduced velocity, and prolonged immobility in zebrafish, indicative of anxiety-like behaviour.	7
THC: 0.024, 0.12, 0.6 mg/L CBD: 0.006, 0.03, 0.15 mg/L, from 6 to 96 hpf, exposure	Larvae Tg(fli1:egfp) zebrafish (96 hpf)	Light-dark test	30 min	Locomotor activity	In F0 larvae, 0.024 mg/L THC significantly reduced locomotor activity during the dark phase, while CBD had no effect. In F1 larvae, 0.12 mg/L THC increased dark-phase activity, whereas 0.024 and 0.6 mg/L had no effect. CBD exposure (0.03 mg/L) in parents led to reduced dark-phase activity in F1 offspring.	8
THC: 0.024, 0.12, 0.6 mg/L CBD: 0.006, 0.03, 0.15 mg/L, from 6 to 96 hpf, exposure THC(F1): 0.6 mg/L CBD(F1): 0.006 and 0.03 mg/L		Open field test	6 min	Total distance traveled Time in center Freezing frequency Freezing duration.	Conversely, in the adult open field test there were no significant effects in F0 fish, but a significant reduction in the time in periphery was seen in F1 fish from the highest THC exposure group	
THC: 0.001 to 20 mg/L, from 5.25 to 10.75 hpf during gastrulation, exposure	Adult Wild type (8 months)	Open Field Test	10 min	Distance moved Time spent in three virtual zones – center, thigmotaxis, transition	THC (0.001–0.5 mg/L) showed no significant differences in distance travelled or zone preference. However, variability in zone time was reduced at 0.5 mg/L. Concentrations ≥ 1 mg/L were not survivable to adulthood.	9
CBD: 1.5, 2, 3 μ g/ml, 0.5 to 10 hpf during gastrulation, exposure THC: 2,3,4,6 μ g/ml, 0.5 to 10 hpf during gastrulation, exposure	Larvae CaMPARI transgenic outcross with Casper strain (5 dpf)	Visual Motor Response (VMR) test	1 hour	Neural activity Locomotor activity	Embryonic CBD exposure (≥ 1.5 μ g/mL) reduced neural activity and locomotion in a dose-dependent manner; effects at 3 μ g/mL were significant, suggesting CNS depression consistent with anxiety-like responses. Embryonic THC exposure (≥ 4 μ g/mL) significantly reduced neural activity and locomotion, with strongest suppression at 6 μ g/mL, consistent with CNS depression and anxiety-like or sedative effects.	10
CBD: 0.1, 0.5, 5, 10 mg/kg, intraperitoneal injection	Adult Wild type (4 months)	Novel Tank Diving Test		Total distance (m) Max speed (m/s) Mean speed (m/s) Absolute turn angle (degrees) Time spent in upper zone	CBD induced an inverted U-shaped effect on anxiety, with 0.5 mg/kg significantly increasing time spent in the upper zone (58% vs. 25% in controls; $p = 0.0002$), indicating anxiolytic effects. Lower (0.1 mg/kg) and higher doses (5–10 mg/kg) showed no significant effect. Locomotor parameters remained unchanged across all doses.	13

Table 1. Anxiety behavioral assay for cannabis-induced zebrafish. (Continued).

Cannabis Treatment	Zebrafish Stage	Behavioural Assay	Test Duration	Parameters	Findings	Ref.
CBD: 0.5, 1, 5, 10 µg/mL, 30 min, ;exposure	Larvae Wild type (4-5 dpf)	Repeated light- dark test	3 hours (15-min interval for each light and dark)	Locomotor activity in dark zone (distance moved, movement speed)	CBD significantly affected locomotor activity with 10 µg/mL reducing movement ($p < 0.05$). Activity was higher at 5 and 10 µg/mL compared to 0.5 µg/mL. After 24 h withdrawal, responses at 0.5 and 1 µg/mL recovered, while 5 and 10 µg/mL showed nonsignificant increases. The 10 µg/mL group had the weakest dark response ($p < 0.01$), which also recovered.	14
THC: 0.024, 0.12, 0.6 mg/L, from 6 to 96 hpf, exposure	Adult Wild type (30 months)	Open Field Test (OFT)	5 min	Distance and swimming speed, mobility, and time spent in the center (inner 50% of the arena) and periphery (outer 50% of the arena)	No significant effect of early-life THC exposure was observed in aged zebrafish.	15
THC: 30 and 50 mg/L, 20 min, exposure	Adult Wild type short fin (5-8 months)	Novel tank test	6 min	Latency to enter top zone Time spent in top zone Number of top zone transition Number of freezing bouts Duration of freezing bouts (s)	Acute THC exposure significantly increased latency to the top and reduced top transitions and duration ($p < 0.005$, $p < 0.05$). Distance and velocity also decreased ($p < 0.05$), indicating hypolocomotion. Unchanged freezing and erratic swimming suggest anxiety-like reduced exploration.	16
THC: 0.05, 0.1, 0.5, 1.5, 2.0µM, CBD: 0.75, 1.0, 1.75, 2.5, 3.75 µM, both 3-4 min, exposure	Larvae AB/wild type (120 hpf)	Light-dark test	3 hours (5-min interval for each light and dark)	Total distance traveled per larva in 60-second intervals Difference in distance traveled before vs. after light-to-dark transition	THC-treated larvae exhibited lower absolute peak activity compared to controls at all concentrations (0.05–2 µM); however, normalized activity changes showed no significant differences from controls. In contrast, CBD reduced the transition response starting at 1.75 µM, with a marked decrease to less than 10% of control levels at 3.75 µM.	17

Table 2. Learning and memory behavioral assay for cannabis-induced zebrafish.

Cannabis Treatment	Zebrafish Stage	Behavioural Assay	Test Duration	Parameters	Findings	Ref.
THC: 100 nM, 1 hour, exposure	Adult Wild type (1 years)	Spatial-cognition task (hole-board test)	3 min	Latency to find the baited hole Swimming path length	Zebrafish showed spatial learning and memory by reducing latency and swim path to locate a baited food hole. Acute THC administration significantly impaired memory retrieval, increasing latency and search distance.	11
THC: 100 nM, 300 nM, 3 μ M, 1 hour, exposure		Two-alternative choice task (colour discrimination learning)	Not mention	Number of correct and wrong decisions Decision latency	Low-dose THC (100 nM) had no effect on memory or decision-making. High-dose THC (300 nM–3 μ M) caused hypoactivity and drastically reduced task responses, despite no significant change in accuracy due to low response counts.	
CBD: 0.1, 0.5, 5, 10 mg/kg, intraperitoneal injection	Adult Wild type (4 months)	Avoidance inhibitory task	Not mention	Latency to enter dark compartment	CBD affected memory in a dose- and timing-dependent manner. Pre-training: 0.1 mg/kg increased test latency, indicating preserved memory, while 0.5 and 10 mg/kg had partial effects. At 5 mg/kg, memory was impaired ($p = 0.0423$). Post-training: 0.1 and 10 mg/kg reduced latency, suggesting impairment, whereas 0.5 mg/kg preserved memory. 5 mg/kg post-training dose impaired memory retention ($p < 0.0001$).	13
THC: 100 nM in 2.5 L of tap water, 1 hour, exposure *For acute, drug treated once, while for chronic, drug treated daily with same procedure	Adult Wild type (1 years)	Active Avoidance Test	30 sec	Avoidance response before shock Escape response after shock Freezing behaviour Percentage of avoidance responses	Acute THC had no significant effect on avoidance learning ($p = 1.0$). Chronic THC impaired learning, with fish failing to reach the criterion and showing inconsistent avoidance responses. Significant group differences were observed, with THC-treated zebrafish performing significantly worse than the Rimonabant group but comparably to controls. Indicate chronic THC exposure impairs avoidance learning.	18
THC: 100 nM in 2.5 l of tap water, 1 hour, exposure	Adult Wild type (1 years)	Pavlovian fear conditioning paradigm	30 min	Bottom-dwelling Erratic movement Freezing behavior	Pre-test showed significant differences in vertical swimming distribution, with fish preferring surface and bottom layers over the mid-zone. During memory retrieval, bottom-dwelling behavior was reduced under red light compared to white light. No significant changes were observed in erratic movements.	19

Table 3. Social interaction behavioral assay for cannabis-induced zebrafish.

Cannabis Treatment	Zebrafish Stage	Behavioural Assay	Test Duration	Parameters	Findings	Ref.
CBD: 5 mg/kg, peritoneal injection (IP)	Adult Wild type (4 months)	Mirror biting test	1 min	Time spent in the zone near the mirror Biting/Sprinting/Changes in colour pattern	CBD at 5 mg/kg did not significantly affect aggressive behavior ($p = 0.2902$), indicating no measurable impact on aggression in zebrafish at this dose.	13
		Social preference test	10 min	Time spent in the side with the stimulus fish	CBD at 5 mg/kg did not significantly affect social interaction behavior in zebrafish ($p = 0.6675$), indicating no alteration in sociability at this dose.	
CBD-rich oil, THC-rich oil, 15 days, exposure	Adult Wild type	Social preference test	Not mention	Time in the conspecific segment Time in the empty segment Latency to enter the conspecific segment	THC-rich oil increased time in both conspecific ($p < 0.001$) and empty segments ($p < 0.05$), while CBD-rich oil also increased conspecific time ($p < 0.05$), indicating enhanced sociability.	20

of studies rely on acute exposure designs, with few investigating the long-term or developmental impact of chronic cannabinoid exposure. Another important limitation is the lack of studies investigating the effects of crude cannabis extracts, which include combined cannabinoids (THC, CBD, minor cannabinoids), terpenes, and flavonoids. This phenomenon, often referred to as the “entourage effect,” may significantly influence neurobehavioral outcomes but remains underexplored in zebrafish models. Sex-based analyses are also frequently overlooked, despite emerging evidence that males and females may respond differently to cannabinoid treatment. Lastly, although zebrafish offer a high-throughput and genetically tractable model, translating findings to human cannabinoid pharmacology must be approached with caution due to species-specific differences in neuroanatomy and metabolic processing.

4. CONCLUSION

This narrative review synthesizes current findings on the behavioral effects of THC and CBD in zebrafish, with a focus on anxiety, cognition, and social interaction. THC is consistently shown to impair cognitive and exploratory behavior, while CBD demonstrates anxiolytic properties at moderate doses. Zebrafish have proven to be a practical and effective model for cannabinoid research due to their conserved endocannabinoid system and suitability for behavioral assays. However, inconsistencies in study design and the lack of mechanistic depth highlight the need for more robust and standardized approaches. Overall, zebrafish remains a promising tool for preclinical screening of cannabinoid effects on the nervous system.

5. FUTURE VIEW

Future research should expand behavioural investigations to include lesser-studied cannabinoids such as CBG, CBN, and CBC, which remain underexplored despite their emerging pharmacological significance. More studies are needed to evaluate their specific effects on anxiety, cognition, and social behaviour in zebrafish. Incorporating sex-based analyses will be essential to identify sexually dimorphic responses, which have been suggested but not consistently addressed in current literature. Longitudinal designs assessing the long-term impact of early-life and chronic exposure will provide critical insights into developmental sensitivity to cannabinoids. Furthermore, integrating behavioural assays with molecular approaches such as gene expression profiling, neurotransmitter assays, and neuroimaging will help elucidate the mechanisms underlying cannabinoid-induced effects. Attention to cannabinoid

pharmacokinetics in zebrafish will enhance translational relevance. Finally, standardizing experimental procedures and improving data reproducibility are crucial steps toward establishing zebrafish as a reliable and scalable model for cannabinoid neuroscience research.

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Author contribution

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