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Effects and interactions of nicotine, alcohol, and marijuana tea extract on zebrafish behavior: An analysis of shoaling coordination, anxiety, escape responses, and sedation

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Abstract

The co-abuse of nicotine, alcohol, and marijuana presents complex health and behavioral challenges due to their diverse effects on the central nervous system. This study investigates the interactions between these substances using zebrafish (Danio rerio) as a model organism, focusing on behavioral changes such as swimming coordination, anxiety, and escape responses. Nicotine, alcohol, and marijuana tea extract (MTE) were administered to zebrafish, and their effects were analyzed through various behavioral assays, including total distance traveled (TDT), discrete swim velocity (DSV), and caudal fin flickering (CFF).

Results revealed that nicotine treatment increased swimming speed and escape behavior while reducing swim coordination. Alcohol showed dose-dependent effects: low concentrations reduced anxiety and increased activity, whereas higher concentrations led to sedation and impaired coordination. MTE treatment resulted in heightened anxiety and reduced activity at high doses, likely due to the psychoactive effects of tetrahydrocannabinol (THC).

Co-treatment studies indicated complex interactions: nicotine combined with low alcohol concentrations exacerbated anxiety, while higher alcohol doses resulted in reduced escape behavior and coordination. Nicotine's impact was partially mitigated by MTE, which shifted TDT and DSV patterns to lower velocities. CFF analysis showed that nicotine increased frequency and amplitude of fin flickering, which was modulated by alcohol and MTE in a concentration-dependent manner.

These findings highlight how nicotine, alcohol, and marijuana interact to influence zebrafish behavior, providing insights into the broader implications for understanding substance abuse. The study underscores the importance of considering combined substance use in therapeutic strategies and behavioral research, revealing how such interactions can modulate or mitigate individual substance effects.

Keywords: Zebrafish; Nicotine; Alcohol; Marijuana; Behavior

1. Introduction

The co-abuse of multiple psychoactive substances, including nicotine, alcohol, and marijuana, presents significant health and societal challenges due to their complex interactions at the neurochemical level. Each of these substances affects neurotransmitter systems in the brain in distinct ways, and their combined use can lead to exacerbated addictive behaviors, cognitive impairments, and a range of health complications.

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1.1. Nicotine and Alcohol

Nicotine and alcohol are both widely used substances, but their effects on the central nervous system are markedly different. Nicotine primarily acts as a stimulant, enhancing dopamine release in the brain's reward pathways, which can reinforce addictive behaviors [1]. Conversely, alcohol generally acts as a depressant, affecting neurotransmitter systems such as the GABAergic and glutamatergic systems, leading to sedation and impaired motor coordination [2]. When used concurrently, nicotine and alcohol can interact synergistically, potentially amplifying each other's effects on dopamine release and increasing the risk of addiction and impaired decision-making [3]. This interaction complicates the therapeutic management of substance use disorders and highlights the need for a nuanced understanding of their combined effects.

1.2. Marijuana and Its Impact on Combined Substance Use

Incorporating marijuana into the use of other substances such as nicotine and alcohol introduces complex interactions affecting both physical and mental health. The key psychoactive ingredient in marijuana, tetrahydrocannabinol (THC), primarily acts on cannabinoid receptor type 1 (CB1) receptors in the brain. These interactions influence neurotransmitter release, impacting mood, cognition, and motor coordination [4-5]. When used with nicotine or alcohol, marijuana's effects can exacerbate cognitive and behavioral impairments. THC may enhance nicotine's addictive potential by altering dopaminergic pathways, increasing the risk of addiction. Additionally, THC's impact on the GABAergic and glutamatergic systems can amplify alcohol's sedative effects, leading to greater motor coordination issues [6-7]. Chronic combined use of these substances heightens the risk of substance use disorders, making it difficult to achieve and maintain sobriety. The unpredictable interactions between marijuana, nicotine, and alcohol increase the risk of overdose and complicate the management of intoxication [8]. Moreover, long-term use can lead to serious health complications. The liver, responsible for metabolizing both alcohol and marijuana, faces an increased risk of disease due to the combined toxic effects. Cardiovascular health may suffer from the interactions between alcohol and nicotine, while mental health issues such as heightened anxiety, depression, and cognitive impairments are further aggravated by marijuana [6]. Overall, the combined use of marijuana with other psychoactive substances creates a complex risk profile, underscoring the need for comprehensive approaches to treatment and intervention.

1.3. Zebrafish as a Model Organism

Recent advances in research have increasingly utilized zebrafish (Danio rerio) as a model organism to study the effects of psychoactive substances. Zebrafish share approximately 70% of their genes with humans, and 84% of human disease-related genes have a zebrafish counterpart, making them a relevant model for studying human diseases and drug effects [9]. The transparency of zebrafish embryos allows for real-time observation of developmental processes, and their rapid development and high reproductive rate make them ideal for genetic and pharmacological studies [10]. Additionally, zebrafish are cost-effective and space-efficient, facilitating high-throughput screening in research [11-12]. Technological advancements such as CRISPR-Cas9 gene editing and advanced imaging techniques have further enhanced the utility of zebrafish in various fields, including neuroscience, toxicology, and drug discovery [13].

1.4. Behavioral Assays in Zebrafish

Zebrafish swimming velocity and patterns are valuable indicators of drug effects, stress responses, and genetic influences on behavior. Changes in swimming speed can provide insights into the impact of psychoactive substances on locomotor activity and neurobehavioral responses [14-20]. Caudal fin flickering (CTF) is another significant behavior studied in zebrafish. This behavior can reflect physiological and neurological processes, indicating stress, environmental changes, or exposure to substances [21].

1.5. Study Objectives

In this study, we aimed to explore the interactions between nicotine, alcohol, and marijuana, specifically focusing on their combined effects. We analyzed changes in total distance traveled (TDT) at each discrete swimming velocity (DSV), swim coordination (sm-cord) among fish, and escape behavior (ES-B). We also assessed the contribution of discrete swimming velocity (DSV) to behavioral changes, using caudal tail flickering (CTF) of immobilized fish with free caudal fin projections. By examining these parameters, we sought to elucidate the complex interactions between these substances and their impact on zebrafish behavior, providing insights into the broader implications for human health and substance abuse research.

2. Material and methods

2.1. Materials

Adult zebrafish (Danio rerio), averaging 3 cm in length and weighing about 700 mg, were sourced from a local aquarium store. Prior to introducing the fish, 3 g of artificial instant ocean salts were added per liter of deionized distilled water for conditioning. This preparation was done on a large scale (200 L) and allowed it to stabilize over three days. Nicotine was obtained from Sigma-Aldrich (CAS Number: 54-11-5), and alcohol from Merck (Product Number: 1009711000). Cannabis plant buds [Product number MAAB5120.1.0RD; (THC 14.4 mg/g)] were supplied by the Cannabis Research Laboratory at The University of the West Indies, Mona Campus. Standardized cannabis tea (MTE) was prepared by boiling the plant buds in water with 0.005% DMSO to aid in solubility, following Hazekamp et al. (2007) [22].

2.2. Zebrafish Maintenance and Selection

Healthy adult zebrafish were selected using specific criteria and kept in large tanks measuring 45×60×25 cm. They were housed in conditioned water with a conductivity of about 1,500 µS/cm and a pH of 7. The temperature was maintained at 28°C, with continuous aeration provided by an air pump to ensure proper oxygen levels. Water quality was managed through daily two-hour filtration and the addition of aquarium disinfectant. Additionally, 30% of the tank water was replaced daily with Millipore-filtered water containing the appropriate amount of instant ocean salt. The fish were fed three times daily (at 10 am, 2 pm, and 6 pm) with bloodworms, enough to be consumed within five minutes. After ten days of maintenance, zebrafish with similar morphology and length, having equal caudal fin length and exhibiting normal swimming and feeding behaviors, were chosen and housed in pairs separately in 1.5 L Yobiisolut mini aquarium tanks for further study.

2.3. Recording of Swimming

Zebrafish swimming was recorded in triplicate using a wide-field camera mounted on a white cardboard box to prevent reflections, positioned directly above the swimming tank. Fish were illuminated with low-intensity white light reflection from beneath the tank. Videos were captured at 12 frames per second using iSpy software. Recordings were made for 20 minutes before treatment, during 20 minutes of exposure to either nicotine (0.5 mg/L, 1.0 mg/L, or 1.5 mg/L), alcohol (0.25% v/v, 0.5% v/v, 1.0% v/v, or 1.5% v/v), or MTE (50 mg/L, 100 mg/L, 150 mg/L, or 200 mg/L). Recording continued for another 20 minutes after 30 minutes of treatment withdrawal.

2.4. Recording of Caudal Fin Flickering

For caudal flickering recording, a single fish at a time was first anesthetized in 400 ml of fish water containing 0.25% phenoxyethanol (v/v). After introducing the fish into the anesthetizing solution, the fish's activity was closely observed. When the fish completely ceased movement even after touching, it was quickly taken out and placed in a sponge-filled clamp in such a way that the soft clamping fastened the fish between the gill and the end of the anal fin, exposing its tail for flickering. The fish's head was capped with a perforated cap attached to the clamp, preventing forward movement of the fish without compromising breathing. The clamp (in the fish's swimming position) was then inserted into the separating bar of a bipartition recording tank, exposing the fish's head in the first chamber fitted with an air supply. The application of drugs was carried out in this chamber. In the second chamber, the caudal fin was projected, and the partition served to prevent air bubbles from the first chamber from entering the second chamber, eliminating air bubble interference during analysis of the recorded video (20 FPS) captured by a webcam fitted on the top of the caudal fin. The webcam was connected to the iSpy program on a computer. Five-minute pre-drug treatment recordings started after 5 minutes of setup to allow full recovery from anesthetization and continued for another 10 minutes after drug treatments. Captured images were processed with NIH ImageJ stack 3D Projection.

2.5. Statistical Analysis

Statistical analyses were carried out using one-way ANOVA

3. Results

3.1. Visual Determination of Swim Coordination, Swim Speed, and Escape Behavior

Videos recorded at 12 fps with iSpy were processed using ImageJ and visualized with Stack3D projection. A representative figure is shown in Figure 1. Control group, zebrafish-maintained swim coordination throughout the 20-minute recording period. In contrast, nicotine-treated fish exhibited increased swimming speed and activity, moving

back and forth across the tank. At a low nicotine concentration (0.25 mg/L), escape behavior increased, with fish continuously striking the tank walls, as seen in lane D of the 0.5 mg/L nicotine-treated fish. Additionally, nicotine treatment not only increased swimming speed and anxiety but also significantly reduced swim coordination. Alcohol-treated fish displayed increased swimming speed at lower concentrations (0.25% v/v), but higher concentrations resulted in decreased swimming speed and coordination. MTE-treated fish showed no change in swim coordination but exhibited a high degree of escape behavior, reduced swimming speed, and swimming cessation at 200 mg/L MTE.

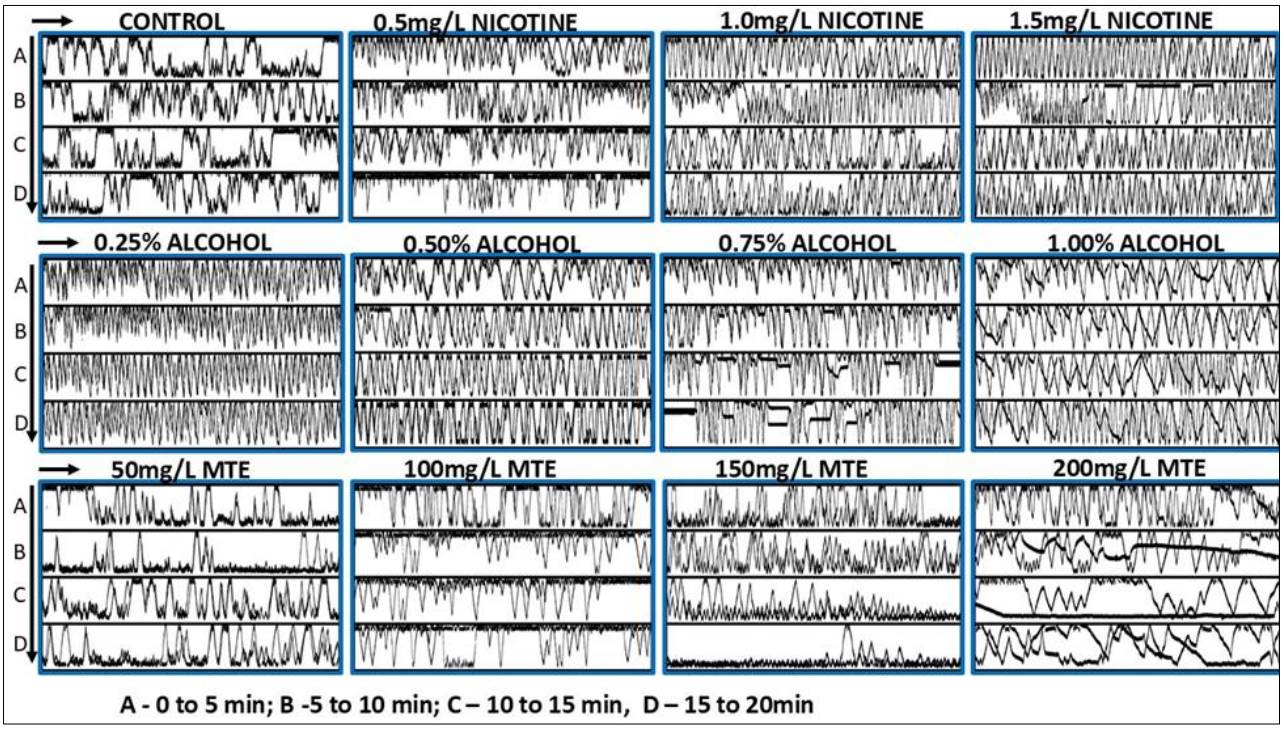

Figure 1 NIH ImageJ 3D Projection of Zebrafish Swimming Under Different Treatment Conditions.

Each box represents a distinct treatment condition, with each row within the box depicting 5 minutes of tracking data. The height of each row corresponds to the length of the swimming tank.

Co-treatment with nicotine (1.0 mg/L) and alcohol (0.25% v/v) led to pronounced escape behavior (Figure 2). Increasing the alcohol concentration (0.5% v/v or above) reduced escape behavior with initial increased but subsequently decreased swimming speed over the treatment duration. Co-treatment with nicotine (1.0 mg/L) and alcohol (1.0% v/v) resulted in highly reduced swimming activity.

In contrast, co-treatment of nicotine (1.0 mg/L) with MTE caused a significant increase in escape behavior at low MTE concentrations. The nicotine-induced increase in swimming speed progressively declined with increasing MTE concentration during co-treatment (Figure 2).

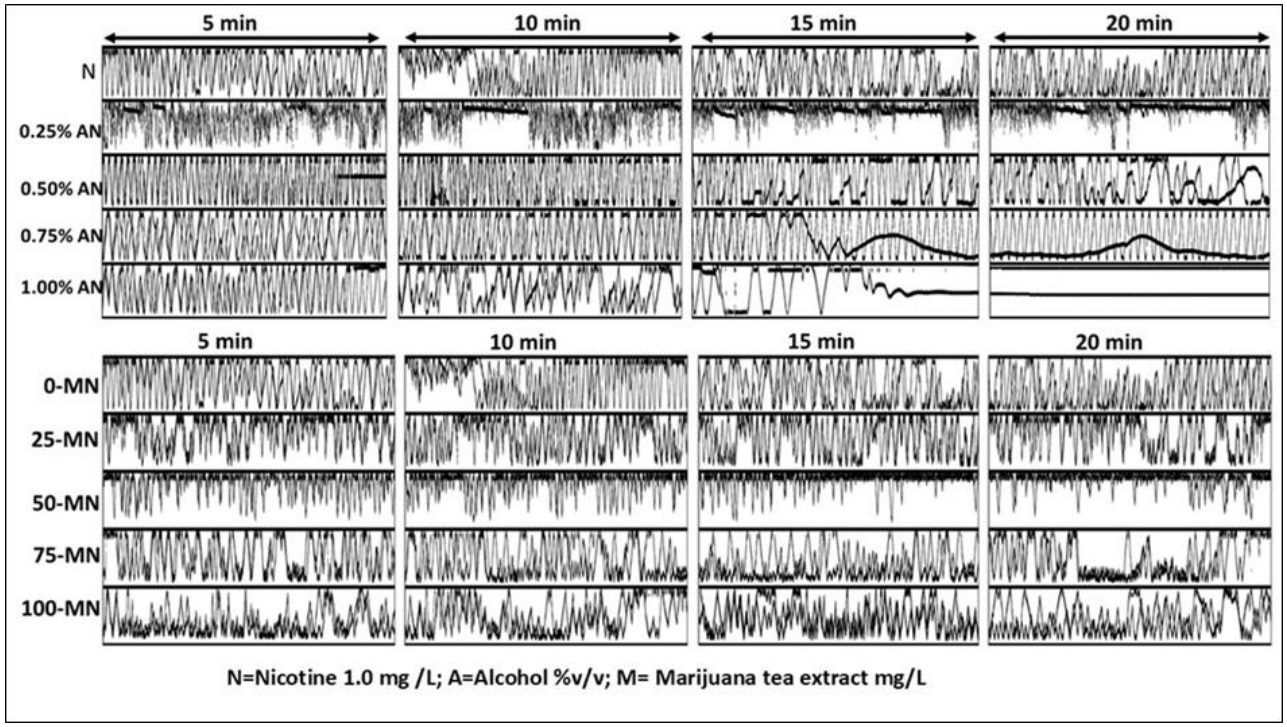

Figure 2 NIH ImageJ 3D Projection of Zebrafish Swimming Under Different Treatment Conditions

Each row represents a distinct treatment condition, and the box represents 5 minutes of tracking data. The height of each row corresponds to the length of the swimming tank. The upper row of boxes displays data for co-treatment with nicotine and varying concentrations of alcohol (0-1.00% v/v), while the lower row shows data for co-treatment with nicotine and varying concentrations of marijuana tea extract (0-100 mg/L).

3.2. Analysis of Discrete Swim Velocity (DSV)

The total distance traveled (TDT) against each DSV plotted in Figure 3. In the control group, the plot exhibited a bell-shaped curve with a peak TDT at a DSV of 4 pixels per second (PPS), which remained consistent over the four 5-minute intervals. Nicotine-treated fish (0.5 mg/L) exhibited a higher TDT at a velocity of 4 PPS compared to the control, with the peak shifting beyond 4 PPS over time. This shift was more pronounced at higher nicotine concentrations (1.0 and 1.5 mg/L). Alcohol-treated fish (0.25% v/v) also showed a shift in the TDT peak beyond DVS of 4 PPS, with greater TDT values throughout the 20-minute recording, but this shift declined with increasing alcohol concentration. MTE-treated fish displayed higher peak TDT at a DSV below 4 PPS, with both DSV values and TDT decreasing with increasing MTE concentration and treatment duration.

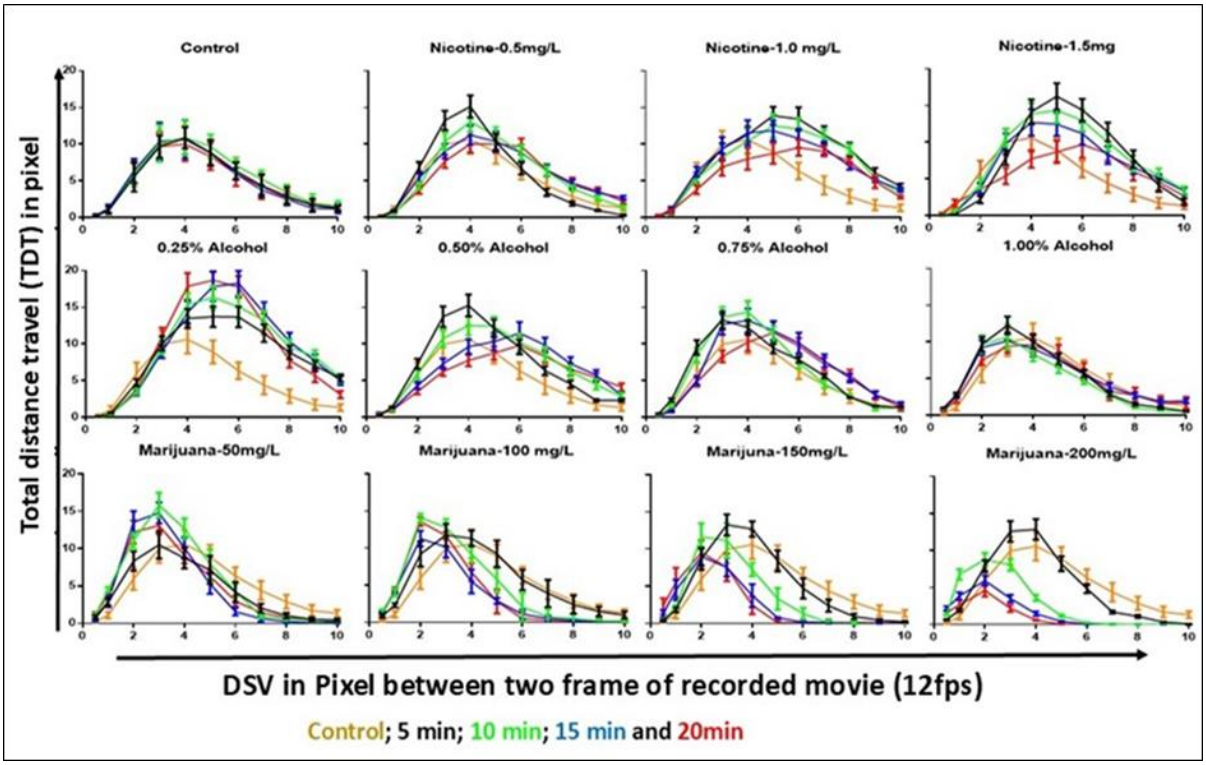

Figure 3 Plot of Total Distance Traveled (TDT) versus Discrete Swim Velocity (DSV) of Zebrafish Under Different Treatment Conditions

Each color curve represents 5 minutes of tracking data: black for the first 5 minutes, green for the second 5 minutes, blue for the third 5 minutes, and red for the final 5 minutes of a total 20-minute tracking period. Each plot shows the tracking values of six fish (Mean ± SD) with three repeats of each experiment involving two fish per repeat.

Co-treatment of nicotine (1.0 mg/L) with various alcohol concentrations (0.25%-1.0% v/v) showed that low alcohol concentrations (0.25% v/v) resulted in higher TDT values at higher DSVs 6 PPS compared to nicotine alone during the initial 5 minutes, but this shifted to lower DSVs over time. The TDT versus DVS curve became smaller with increasing alcohol concentration (Figure 4 top row). Co-treatment of nicotine (1.0 mg/L) with various MTE concentrations (25-100 mg/L) showed that MTE shifted DSV from higher (in the case of nicotine alone) to lower DSVs in a concentration-dependent manner, also reducing TDT in a time-dependent manner (Figure 4 bottom row).

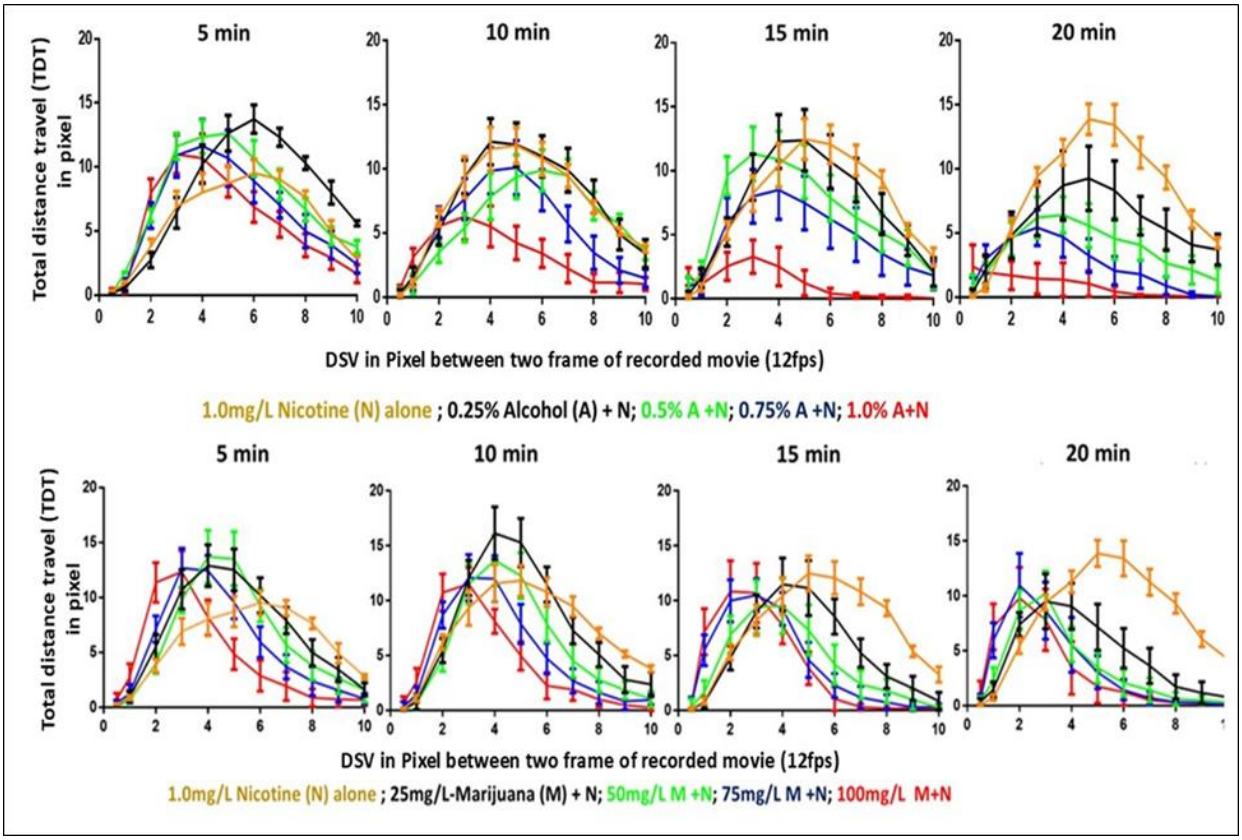

Figure 4 Plot of Total Distance Traveled (TDT) versus Discrete Swim Velocity (DSV) of Zebrafish Under Different Treatment Conditions

Each color curve represents the treatment type. Each plot displays the tracking values of six fish (Mean ± SD) with three repeats of each experiment involving two fish per repeat. The upper row presents data for co-treatment with nicotine and varying concentrations of alcohol (0-1.00% v/v), while the lower row shows data for co-treatment with nicotine and varying concentrations of marijuana tea extract (0-100 mg/L).

Pre-treatment with low MTE concentration (50 mg/L) followed by high MTE concentration (100 mg/L) produced the same shift to lower DSVs compared to the control, but with a higher TDT than treatment with either 50 or 100 mg/L MTE alone (Figure 5 left). In the presence of nicotine (1.0 mg/L), high concentration MTE treatment (100 mg/L) after low concentration MTE pre-treatment (50 mg/L) did not produce a shift or change in TDT at each DSV, indicating nicotine can prevent extreme shifts in swimming behavior induced by marijuana (Figure 5 right).

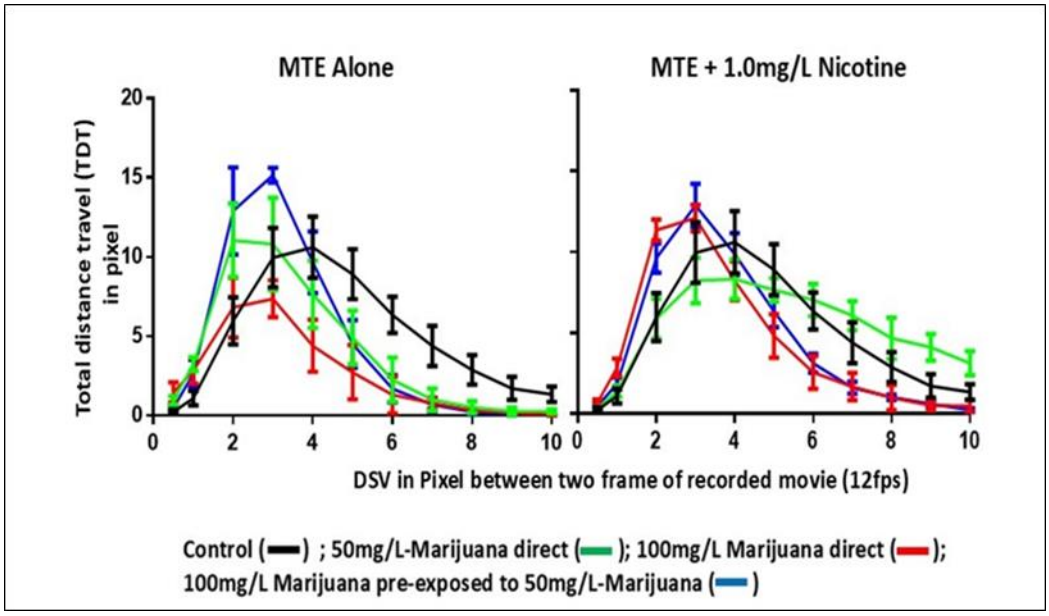

Figure 5 Plot of Total Distance Traveled (TDT) versus Discrete Swim Velocity (DSV) of Zebrafish Showing the Influence of Nicotine on Tolerance to Marijuana Tea Extract

Each color curve represents tracking data from the last 5 minutes of 20 minutes of treatment. Each plot displays the tracking values of six fish (Mean ± SD) with three repetitions of each experiment involving two fish per repetition.

Thirty minutes after drug withdrawal, alcohol-treated fish exhibited higher DSVs and greater TDT values, associated with swim burst behavior. Nicotine-treated fish (1.0 mg/L) showed a peak TDT comparable to the control but with higher TDT values. Co-treatment with nicotine (1.0 mg/L) and alcohol (0.5% or 1.0% v/v) showed that 0.5% alcohol co-treated fish had similar DSV shifts to nicotine alone, while 1.0% alcohol co-treated fish had higher DSVs with similar peak TDT to the control (Figure 6 top row). MTE-alone treated fish had peak TDT shifted to lower DSVs, but co-treatment with nicotine normalized the peak TDT to control levels after drug withdrawal (Figure 6 bottom row).

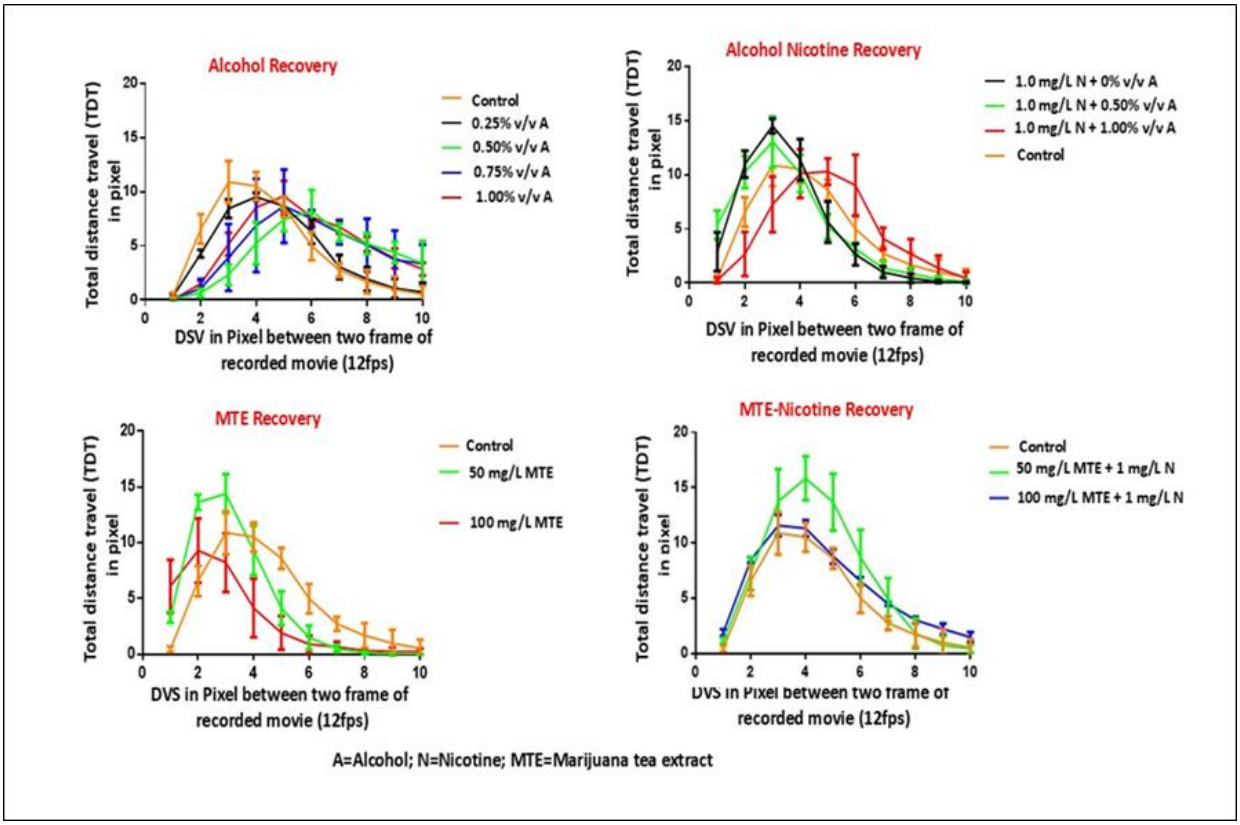

Figure 6 Plot of Total Distance Traveled (TDT) versus Discrete Swim Velocity (DSV) of Zebrafish Showing the Influence of Nicotine on Recovery from Alcohol or Marijuana Tea Extract Intoxication

Each color curve represents tracking data from the first 5 minutes following 30 minutes of recovery from treatment. Each plot displays the tracking values of six fish (Mean ± SD), with three repetitions of each experiment involving two fish per repetition.

3.3. Analysis of Caudal Fin Flickering (CFF)

Stack3D projection of recorded CFF patterns showed that in control fish, the pattern consisted of a slow, longer duration followed by repeating flickering. Alcohol treatment (0.5% v/v) produced small amplitude, moderate frequency wavy CFF, while 1.0% v/v alcohol resulted in large amplitude, high frequency, but short duration wavy CFF, which came to a standstill after 5 minutes. Nicotine treatment (1.0 mg/L) resulted in low amplitude, extremely high-frequency CFF. Co-treatment of nicotine (1.0 mg/L) with increasing alcohol concentrations (0.25%-1.0% v/v) did not diminish or mask the effects of either drug, but nicotine appeared to potentiate the alcohol effect, increasing both amplitude and frequency of CFF compared to alcohol alone.

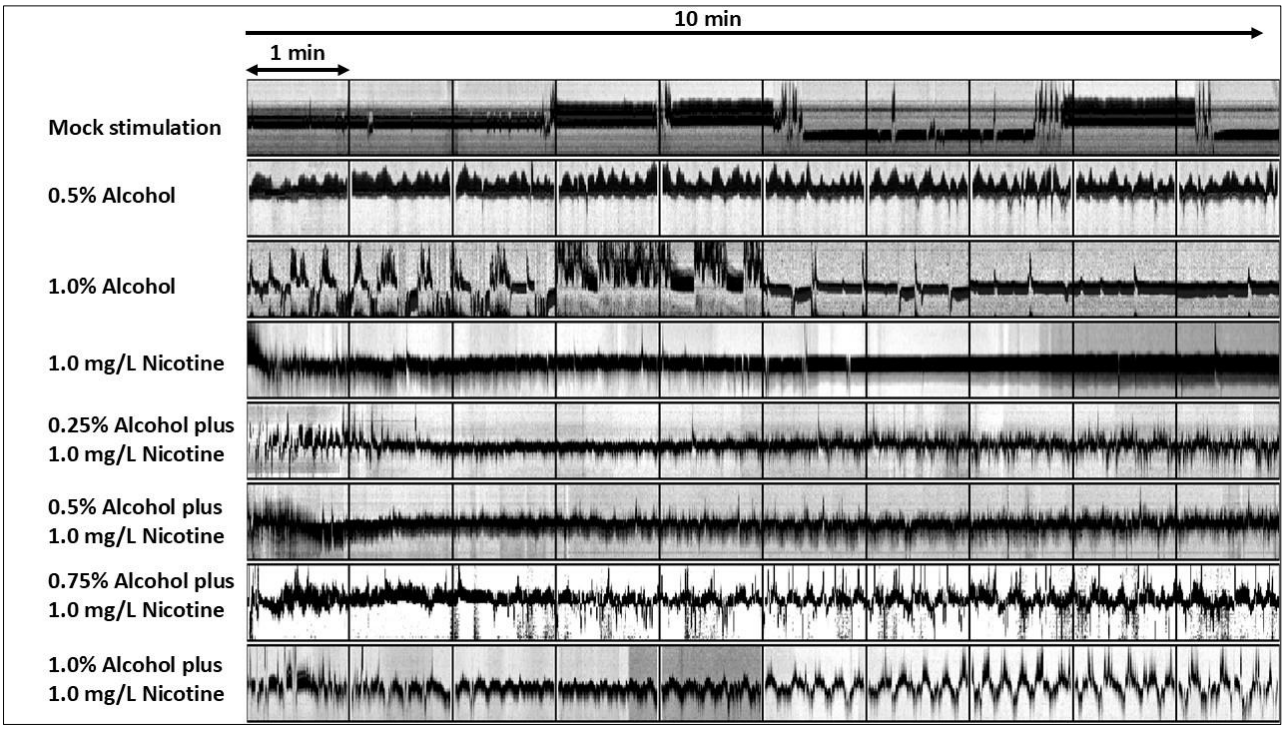

Figure 7 NIH ImageJ 3D Projection of Zebrafish Caudal Fin Flickering (CFF) Under Different Treatment Conditions

Each box represents 1 minute of data, consisting of a 3D stack projection derived from 1200 frames of recorded video. The height of each row within a box corresponds to the caudal fin length. Each row displays data for a specific treatment condition, as indicated on the side of the row.

In contrast, MTE-treated fish exhibited indifferent CFF patterns at low concentrations (50 mg/L MTE), but with increasing MTE concentration and treatment duration, the CFF pattern became slower and appeared to use considerable force, especially at higher MTE concentrations (150 mg/L). Co-treatment of nicotine (1.0 mg/L) with various MTE concentrations (25-100 mg/L) showed that MTE inhibited nicotine-dependent high-frequency CFF in a concentration-dependent manner. Fish also exhibited MTE-dependent CFF patterns at lower MTE concentrations in the presence of nicotine compared to MTE alone.

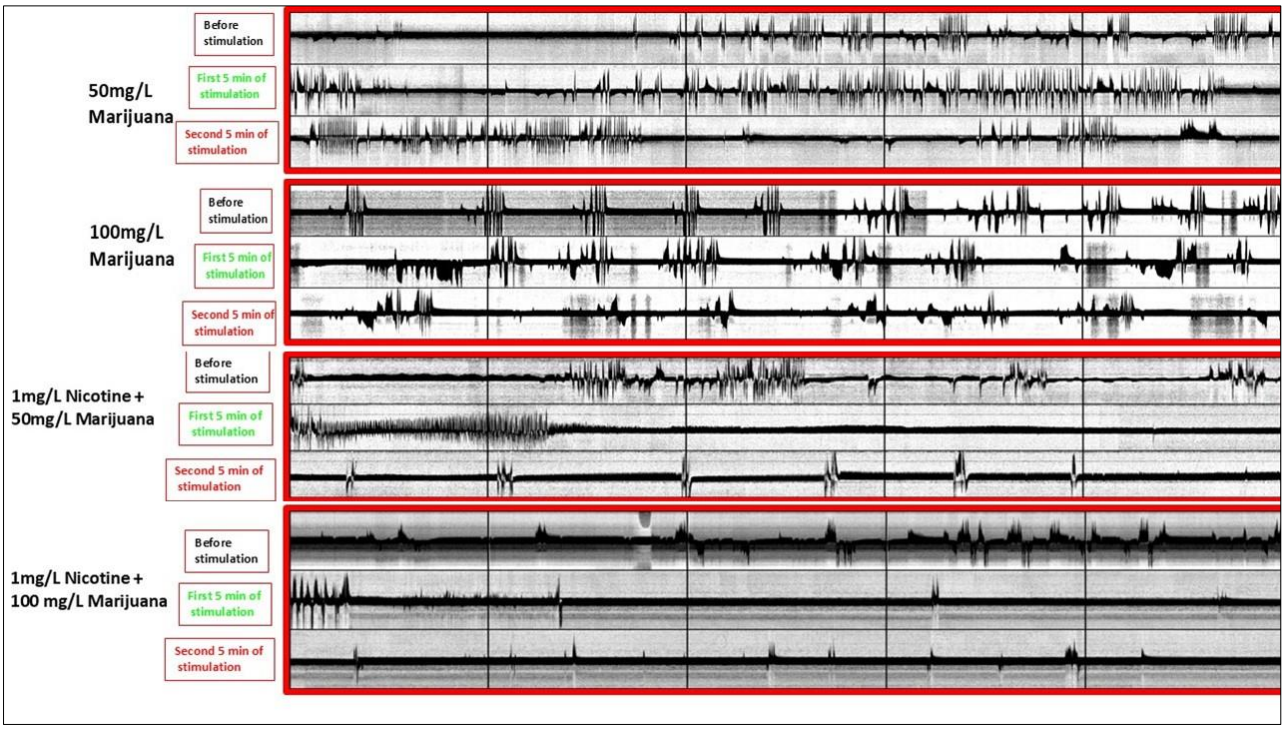

Figure 8 NIH ImageJ 3D Projection of Zebrafish Caudal Fin Flickering (CFF) Under Different Treatment Conditions

Each box represents 1 minute of data, consisting of a 3D stack projection derived from 1200 frames of recorded video. The height of each row within a box corresponds to the caudal fin length. Each row displays data for a specific treatment condition, as indicated on the side of the row.

4. Discussion

4.1. Swin co-ordination

Zebrafish shoaling is a well-established behavior in which fish maintain close proximity or continuously adjust their positions relative to one another to ensure group cohesion. This behavior is guided by visual cues and is regulated by the habenula, a brain region involved in decision-making and social interactions [23-24].

Visual analysis using ImageJ Stack3D projection of recorded videos revealed that untreated control zebrafish exhibited optimal shoaling behavior, characterized by close proximity and coordinated movement without aggressive interactions. In contrast, exposure to nicotine, alcohol, MTE, or combinations of these substances (nicotine with alcohol or nicotine with MTE) resulted in a marked reduction in shoaling behavior, with the degree of disruption varying according to the drug concentration. These findings suggest that all tested psychoactive substances interfere with visual cues and social interactions, impairing the zebrafish's ability to maintain cohesive group behavior.

4.2. Anxiety, escape behavior and sedation

Zebrafish (Danio rerio) have become a key model organism for studying anxiety and escape behaviors, providing valuable insights into vertebrate psychology and neurobiology. Anxiety in zebrafish is typically assessed through behavioral assays that measure their responses to various stressors and their tendencies to avoid or approach different stimuli [18]. One such behavior is "striking," where zebrafish swim forcefully toward the tank walls, often as a response to perceived threats or stress [25]. This behavior can be experimentally induced through various stressors, including visual, auditory, or chemical stimuli, and is quantified by metrics such as the frequency, duration, and intensity of strikes [26].

Our analysis demonstrated that nicotine at a low concentration (0.5 mg/L) produced an anxiogenic effect, characterized by increased anxiety and escape behavior. As the concentration increased (1.0-1.5 mg/L), the effect shifted to anxiolytic, with heightened swimming speed and frequent sudden stops, consistent with previous observations [14]. At the highest concentration (2.0 mg/L), the frequent sudden stops may be attributed to nicotine-induced respiratory depression [27].

Similarly, alcohol at low concentrations (0.25% v/v) exhibited anxiolytic effects, reducing anxiety and stress through GABA-A receptor modulation [28-29]. Higher alcohol doses resulted in sedation and reduced motor coordination, which aligns with the activation of the GABAergic system and inhibition of the glutamatergic system [30].

In MTE-treated fish, anxiety-like swimming behavior was observed across all concentrations (50-150 mg/L), although high doses (200 mg/L) led to reduced activity after an initial period of increased movement. CBD, a non-psychoactive cannabis component, has been shown to possess anxiolytic properties through interaction with the 5-HT1A serotonin receptor [31-32]. In contrast, THC, the primary psychoactive component of cannabis, can induce acute anxiety responses, particularly at high doses or in inexperienced users [33]. Analysis of cannabis oil extracts revealed 14.4% THC and negligible cannabidiol, suggesting that high anxiety levels could be attributed to THC, although the role of terpenes cannot be excluded. High doses of THC have been associated with increased anxiety, panic attacks, and paranoia [33]. Chronic anxiety in zebrafish often results in reduced swimming activity and increased immobility [18].

Co-treatment with nicotine (1.0 mg/L) and varying concentrations of alcohol (0.25-1.0% v/v) showed that low alcohol concentrations produced a strong anxiogenic response, while higher concentrations had anxiolytic effects, reducing the timing of sedative-like behavior with increased alcohol concentration. This finding supports earlier observations that alcohol can overshadow nicotine's effects [14]. Conversely, co-treatment with nicotine (1.0 mg/L) and MTE (25-100 mg/L) showed persistent anxiety, with nicotine's effects diminishing at higher MTE concentrations. THC's activation of CB1 receptors in different brain regions affects various aspects of brain function and behavior. For instance, THC impacts the hippocampus, impairing learning and memory processes [34], disrupts motor function and coordination in the basal ganglia [35].

4.3. DSV and CFF Analysis

Control zebrafish demonstrated stable swim patterns over the 20-minute recording period, with no significant changes in the TDT versus DSV plots, indicating a relaxed state without external or internal stimulants. The corresponding CFF analysis showed slow, rhythmic patterns, reflecting steady swim coordination.

In contrast, nicotine treatment led to high-frequency, low-amplitude CFF patterns and a shift in the peak TDT to higher DSV values, which changed continuously over time. This aligns with previous findings that fast CFF is associated with increased swimming speed [36]. Low concentrations of nicotine (0.5 mg/L) produced an anxiogenic effect, while higher concentrations (1.0-1.5 mg/L) resulted in increased swimming speed and frequent sudden stops, suggesting a shift to an anxiolytic effect. However, at very high nicotine concentrations (2.0 mg/L), the frequent sudden stops may be due to nicotine-induced respiratory depression [27].

Alcohol treatment at low concentrations (0.25% v/v) led to fast, low-amplitude wavy CFF patterns, with amplitude increasing briefly before becoming very slow after 10 minutes. This is supported by the TDT plots, which showed a shift to lower DSV values, indicating reduced swimming speed. Such wavy CFF patterns are associated with slower swimming [37].

Co-treatment with nicotine and alcohol (0.25-1.0% v/v) caused a pronounced shift in the TDT peak to lower DSV values compared to nicotine alone, reflecting the combined effects of both substances. The CFF analysis revealed a combination of nicotine and alcohol effects, with trembling and wavy fin motion leading to reduced swimming speed [38-39].

MTE treatment resulted in a concentration-dependent shift of the TDT peak to lower DSV values, with peak values decreasing compared to control. At low MTE concentrations (50 mg/L), CFF patterns were relatively stable, but higher concentrations (150 mg/L) led to slower CFF patterns and increased difficulty in fin movement, resulting in reduced swimming efficiency [37]. Co-treatment with nicotine (1.0 mg/L) and various MTE concentrations (25-100 mg/L) showed that MTE inhibited nicotine-induced high-frequency CFF in a concentration-dependent manner. This is consistent with findings that THC reduces the number or sensitivity of nAChRs, thus diminishing nicotine's ability to activate these receptors or modulate neurotransmitter release [40-41].

Despite the shift in TDT peaks to lower DSV values with MTE, the TDT curve was more uniform compared to MTE alone, suggesting that nicotine partially mitigates extreme shifts induced by MTE. This aligns with observations that co-administration of nicotine with THC alters cognitive performance and enhances the rewarding properties of THC [41]. Pre-exposure to low MTE concentrations (50 mg/L) followed by higher concentrations (100 mg/L) led to increased anxiety, with TDT versus DSV plots showing shifts to lower DSV values and higher TDT values. However, these shifts and peak values were stabilized when nicotine was co-administered, supporting the concept that nicotine can enhance tolerance and stabilize the effects of THC [41].

Co-treatment with alcohol and nicotine also appeared to prevent excessive swimming bursts and anxiety-like behaviors associated with alcohol withdrawal, as well as slow swimming tendencies after withdrawal from MTE-nicotine co-treatment. Nicotine's effects on the dopaminergic system are thought to alleviate mood disturbances related to alcohol withdrawal by increasing dopamine levels, counteracting deficits observed during withdrawal. Similarly, nicotine can help mitigate cannabis withdrawal symptoms through modulation of cannabinoid receptor activity [42].

5. Conclusion

This study explores the effects of nicotine, alcohol, and MTE on zebrafish behavior, focusing on shoaling coordination, anxiety, escape responses, and sedation. Untreated control zebrafish exhibited optimal shoaling and movement, while exposure to these substances disrupted coordination and social behavior. Nicotine and alcohol showed dose-dependent impacts on anxiety and escape responses, with low doses reducing anxiety and higher doses causing sedation and impaired motor coordination. MTE consistently induced anxiety-like behavior, with high doses leading to reduced activity, likely due to THC's psychoactive effects. Co-treatment revealed complex interactions: low alcohol exacerbated anxiety, while higher doses had anxiolytic effects. Nicotine's impact was partially mitigated by MTE. Analysis of swimming patterns (DSV) and CFF patterns showed significant alterations due to substance exposure, highlighting how combined substance effects can modulate or mitigate individual impacts. This research enhances understanding of psychoactive substance interactions and their behavioral implications.

Compliance with ethical standards

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Authors' Contribution

Mohammad Kutub Ali: Research supervisor and coordinator, data analyser and manuscript drafting; Chevaughn Okeive Witter: Swimming data collection; Victoria Johneive Richards and Brandy Moesha Miller: Caudal Fin Flickering data collection.

Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

Ethical approval was granted by the Mona Campus Research Ethics Committee (Ref: AN4, 19/20 and CREC-AN.004,2022/2023).

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