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Developmental exposure to minor cannabinoids causes morphological and behavioral adverse outcomes in zebrafish larvae

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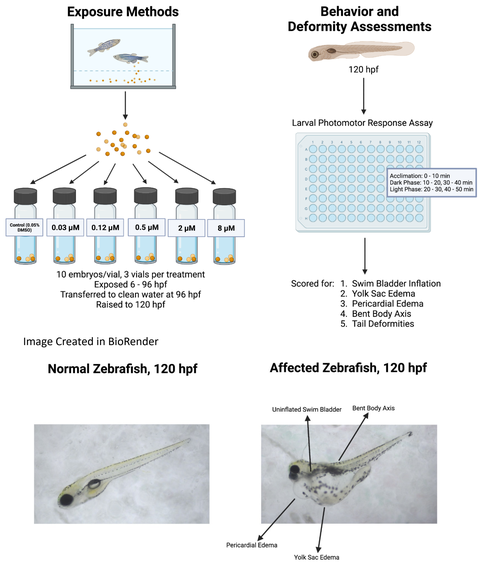
Introduction

- Minor cannabinoids are naturally occurring minor components of the cannabis plant.
- With recent large-scale legalizations of cannabis for recreational use, minor cannabinoids have become increasingly used.
- Cannabinoids are even marketed to pregnant women to relieve symptoms, but there is little research in their safety for a developing fetus.

Objectives

- Determine if minor cannabinoid exposure results in adverse morphological and behavioral effects in developing zebrafish as was previously measured following THC and CBD exposures
- Understand relative potency of the different cannabinoids for developmental toxicities

Methods



Δ8-Tetrahydrocannabinol (Δ8THC) and Tetrahydrocannabivarin (THCV) Results

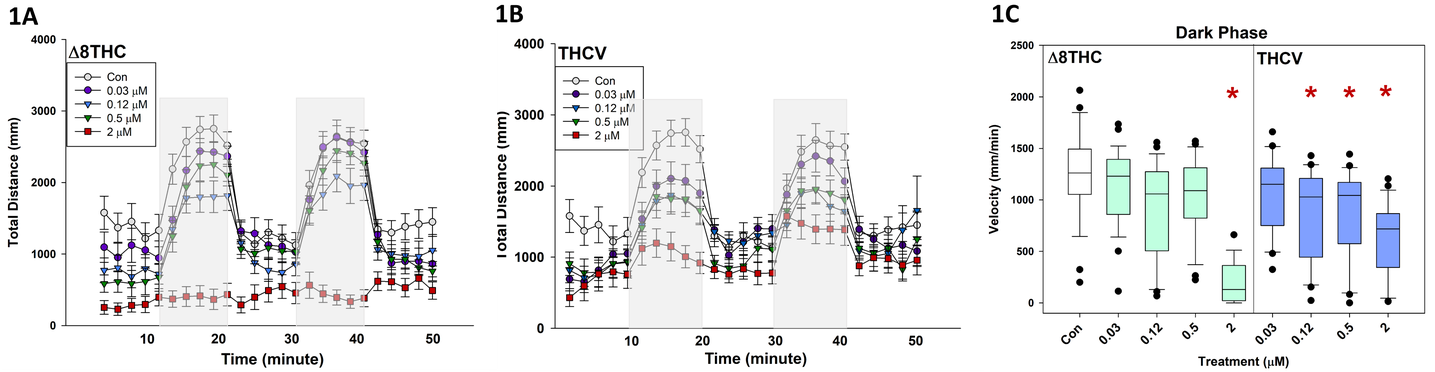


Figure 1A-B: Distance traveled by Δ8THC (**1A**) and THCV (**1B**) treated fish over 50 min (0-10 min acclimation, 10-20 and 30-40 min dark, 20-30 and 40-50 min light). Δ8THC (2 µM) and THCV (0.12, 0.5, and 2 µM) treated fish were hypoactive during the light (Δ8THC) and dark phases (Δ8THC and THCV).

Figure 1C: Δ8THC (2 µM) and THCV (0.12, 0.5, and 2 µM) treated fish had significant (*) hypoactivity compared to the controls (ANOVA on ranks; Dunn's posthoc test; *p≤0.05).

Cannabigerol (CBG) and Cannabinol (CBN) Results

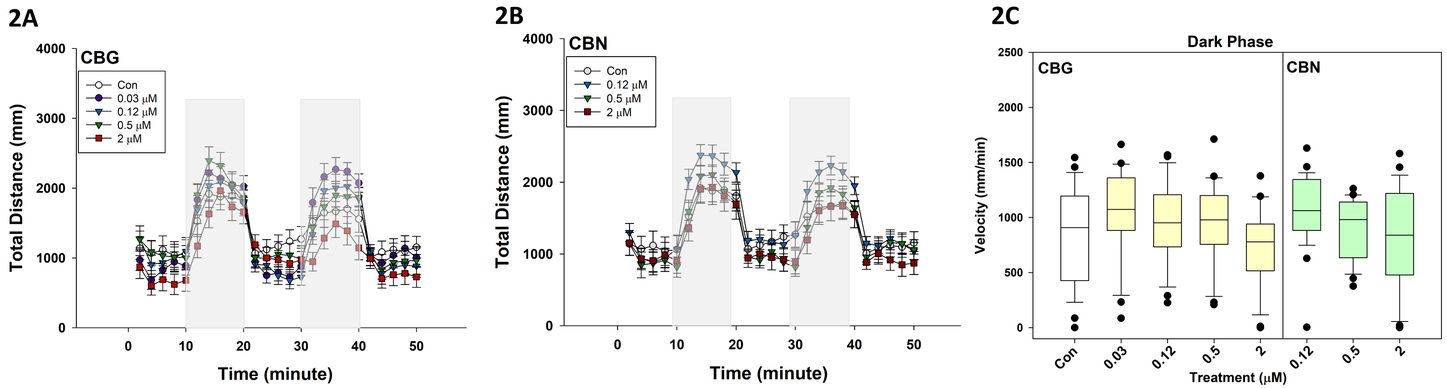


Figure 2A-B: Distance traveled by CBG (**2A**) and CBN (**2B**) treated fish over 50 min (0-10 min acclimation, 10-20 and 30-40 min dark, 20-30 and 40-50 min light). Behavior of treated fish did not significantly deviate from the control fish.

Figure 2C: Neither CGB nor CBN exposure caused significant effects on larval behavior during the dark phase (**2C**), light phase or acclimation phase (data not shown; ANOVA on ranks).

Results

Mortality:

- All four of the minor cannabinoids were 100% lethal at the highest concentration of 8 µM: THCV and CBG at 48 hpf; and Δ8THC and CBN at 96 hpf.

Morphological Changes:

- While present in some fish, adverse morphological changes were not statistically significant compared to control-treated fish.

Behavioral Changes:

- Behavior was significantly altered following Δ8THC and THCV exposure (Figure 1) but not CBG or CBN exposure (Figure 2).

Conclusions

- These results suggest exposure to minor cannabinoids can, like Δ9-THC and CBD, cause significant behavioral changes in developing zebrafish.
- Differences between cannabinoid effects could be attributed to differences in chemical structure, bioavailability, and/or cannabinoid receptor 1 and 2 binding affinities (**Table 1**).
- The human relevance of developing toxicity of minor cannabinoids needs further consideration.

Table 1: The cannabinoids that have been (**bolded**)/will be screened in this study including abbreviations and representative CB1 Ki (nM) and affinity (unk = unknown).

Cannabinoid		CB1 Ki	Affinity	Ref
Δ9-tetrahydrocannabinol	THC	18	agonist	1
Cannabidiol	CBD	151	weak agonist	1
Δ8-tetrahydrocannabinol	8-THC	78	partial agonist	1
Tetrahydrocannabivarin	THCV	22	antagonist	1
Cannabigerol	CBG	3090	partial agonist	1
Cannabinol	CBN	75	agonist	1
Cannabidiolic acid	CBDA	626	Aginverse agonist	2
Tetrahydrocannabinolic acid	THCA	1292	+ allosteric mod	1
THC acetate ester	THCO	unk		
Tetrahydrocannabiphorol	THCP	1.2	agonist	3
αHexahydrocannabinol	HHC	117	agonist	1
Δ10-tetrahydrocannabinol	10-THC	unk		

1. Husni AS, et al. *Med Chem Res*. 2014;23(9):4295-4300.
2. Navarro G, et al. *Pharmacol Res*. 2020;159(May):104940.
3. Citti C, et al. *Sci Rep*. 2019;9(1):1-13.

Future Experiments

- Six more minor cannabinoids (CBDA, THCA, THCO, and THCP, Δ10THC, and HHC) will be tested for behavioral and morphological adverse outcomes.
- We will also determine cannabinoid exposure impacts on brain mitochondrial energetics.