Assessing the Developmental Neurotoxicity of Perinatal Exposure to Cannabidiol in Sprague Dawley Rats

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Introduction

- CBD is a compound found in cannabis.
- Epidiolex is the only FDA-approved use of CBD and is used to treat rare childhood seizure conditions.
- There is a large market of unregulated CBD-containing products, which is estimated to reach approximately $20 billion in sales by the year 2025.
- These CBD products are commonly used by the general public to purportedly treat inflammation, pain, and anxiety; which are also all negative symptoms of pregnancy.
- CBD is often marketed as a "safe, naturally occurring" compound, thus there is a significant likelihood of CBD use during pregnancy; however, there is currently inadequate research into its safety and efficacy.
- Cannabis use during pregnancy is linked to poor birth and developmental outcomes, and CBD cannot currently be excluded as a potential contributing factor to these effects.
- This project aims to provide a comprehensive data set to characterize neurobehavioral and neurochemical effects of perinatal CBD exposure using Sprague-Dawley rats.

Methods

- Sprague-Dawley rat dams were orally dosed via gavage once daily with CBD from gestational day (GD) 6 until the day prior to parturition.
- CBD doses include 15, 30, 100, 250, 300, and 350 mg/kg, as well as vehicle control.
- Pups were orally dosed via gavage from postnatal day (PND) 1 until PND 21 with the same dose as the respective dam.
- Brain tissue and plasma were collected at PND 21 and PND 180 for protein and neurochemistry assays.
- Pups were weaned at PND 21 for behavioral testing from PND 22 – PND 180.
- CBD doses for behavior and hormone assays were 15-250 mg/kg. Pup weight, HPLC, and Western blots included a small subset of 300 mg/kg.
- Behavioral tests include tests of motor function, anxiety-like behavior, sensation and perception, and cognitive functions.

Maternal Toxicity at 350 mg/kg

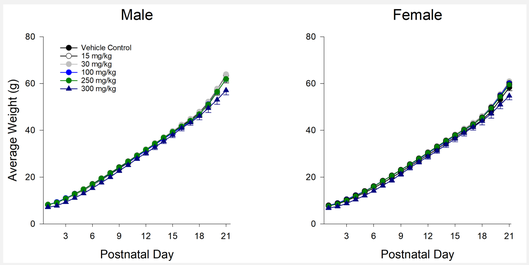
Seven of 7 (100%) underwent unscheduled sacrifice due to excessive weight loss by GD 8. Pathological examination revealed all dams were gravid with non-viable fetuses. Therefore, this dose was discontinued.

Maternal and Fetal Toxicity at 300 mg/kg

Subject	Offspring at Birth	Outcome
607	13 live	Dam killed all pups by PND 1
608	n/a	Necropsy showed non-viable fetuses at GD 19
609	10 live and 3 dead	Pups failed to thrive by PND 2
610	10 live; culled to 8	Dam killed 5 of 8 pups by PND 6
612	11 live and 3 dead; culled to 8	Pups survived to weaning

Five of 10 dams lost offspring and/or met criteria for humane endpoint.

Pup Developmental Weights

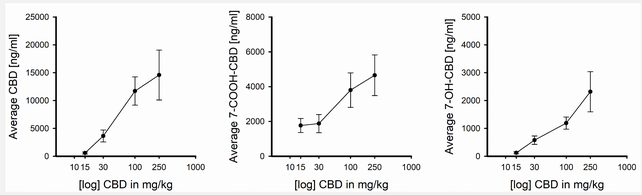


Average pup weights are displayed for male and female rats from PND 1–PND 21.

Due to maternal and fetal toxicity, the 300 mg/kg group was not maintained after PND 21.

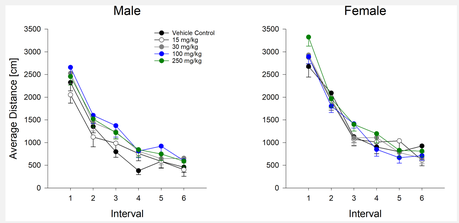
N ≥ 20 litters for all groups, except 300 mg/kg (n=7)

CBD and Metabolite Plasma Concentrations



Plasma was collected from CBD treated PND 21 rats, and CBD and two major metabolites were quantified.

Open Field

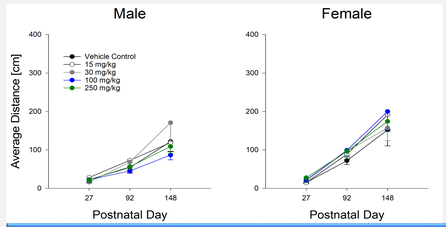


Motor activity was assayed for 30 minutes on PND 26.

Average distance during each 5-minute interval is plotted on the y-axis, with interval number on the x-axis.

N ≥ 19 for all groups.

Rotarod

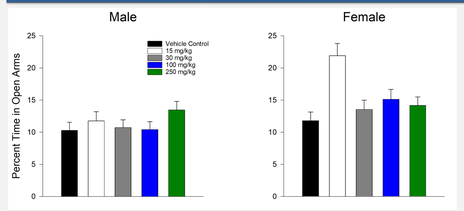


Motor coordination was assayed via accelerating Rotarod at PND 27, 92, and 148.

Average distance on the rotating rod is displayed on the y-axis, with testing day on the x-axis.

N ≥ 20 for all groups.

Elevated-Plus Maze

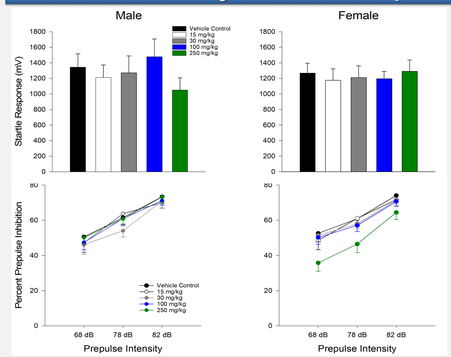


Anxiety-like behavior was assayed via elevated-plus maze on PND 41.

Average percent time spent in the open arms of the apparatus is displayed. More time in open arms is indicative of lower anxiety-like behavior.

N ≥ 20 for all groups.

Auditory Startle & Prepulse Inhibition (PPI)



Startle response and sensory motor gating were measured via PPI on PND 42. Average startle response measured in mV to a 120 dB stimuli are shown in the top panels.

Average percent PPI is shown in the bottom panels to 3 distinct prepulse levels displayed on the y-axis, with the prepulse dB levels on the x-axis.

N ≥ 17 for all groups.

Hormone Panel (ng/ml)

Estradiol

	Males			Females		
Treatment	Mean	SD	N	Mean	SD	N
0	0.051	0.029	8	0.050	0.022	7
15	0.060	0.022	5	0.044	0.013	7
30	0.068	0.010	4	0.037	0.034	7
100	0.045	0.017	4	0.050	0.021	5
250	0.043	0.020	7	0.050	0.010	3

Progesterone

	Males			Females		
Treatment	Mean	SD	N	Mean	SD	N
0	0.051	0.029	8	0.050	0.022	7
15	0.060	0.022	5	0.044	0.013	7
30	0.068	0.010	4	0.037	0.034	7
100	0.045	0.017	4	0.050	0.021	5
250	0.043	0.020	7	0.050	0.010	3

Testosterone

	Males			Females		
Treatment	Mean	SD	N	Mean	SD	N
0	0.93	0.46	8	0.70	0.35	7
15	1.15	0.65	7	0.74	0.34	7
30	1.05	0.57	8	0.61	0.31	7
100	1.15	0.64	8	0.55	0.20	7
250	1.42	0.58	8	0.62	0.23	8

A hormone multiplex kit was run. This assay utilized PND 21 plasma and was run in duplicate. Significant differences include decreased progesterone in the male 250 mg/kg group compared to controls.

Dopamine and Metabolites (ng/g)

Dopamine

	Males			Females		
Treatment	Mean	SD	N	Mean	SD	N
0	1926.5	141.8	7	1966.9	267.6	5
15	1721.7	547.6	7	1839.6	199.4	5
30	1943.8	152.2	5	1887.7	123.8	6
100	1551.0	635.9	6	1714.1	448.4	6
250	1663.4	649.2	5	1738.2	362.1	6
300	2013.6	62.7	4	1850.9	62.8	3

3,4-Dihydroxyphenylacetic Acid

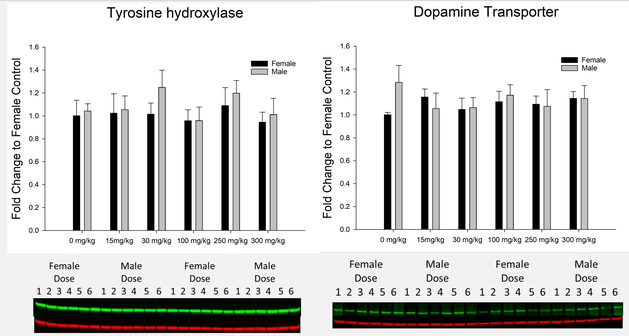
	Males			Females		
Treatment	Mean	SD	N	Mean	SD	N
0	1081.2	258.4	7	942.0	286.5	5
15	918.9	369.2	7	927.9	180.5	5
30	1060.2	419.6	5	886.6	223.8	6
100	877.9	324.1	6	902.1	290.2	6
250	806.4	337.7	5	851.2	163.1	6
300	777.0	142.7	4	1223.6	138.2	3

Homovanillic Acid

	Males			Females		
Treatment	Mean	SD	N	Mean	SD	N
0	977.1	173.0	7	866.3	136.5	5
15	899.3	286.3	7	885.4	165.0	5
30	892.9	161.3	5	828.2	98.2	6
100	904.9	268.7	6	905.6	280.2	6
250	749.3	179.6	5	880.1	118.3	6
300	793.2	52.3	4	838.4	10.6	3

HPLC assays for dopamine and 2 major metabolites were performed on PND 21 striatal tissue.

Striatal TH & DAT



Average fold change relative to female controls for striatal TH and DAT are shown on the left and right, respectively. Striatal tissue was isolated from PND 21 rat brains for 6 CBD treatment groups. Relative protein amount was measured via Western blot and normalized to beta-actin.

Representative blots are displayed below each graph and show bands (red = beta-actin) for all 6 treatment groups in order from Vehicle control (1) to 300 mg/kg (6).

N ≥ 7 for all groups.

Conclusions

- Maternal toxicity was observed at 350 mg/kg.
- Maternal and fetal toxicity was observed at 300 mg/kg.
- Preliminary analysis suggests that very high dose CBD, 300 mg/kg, reduced body weight in pre-weaning pups (PND 19-21).
- Preliminary behavior analyses suggest CBD did not have dose-dependent effects on motor function, anxiety-like behavior, cognition, or memory.
- CBD did not have an effect on plasma levels of developmental hormones, except the highest dose, 250 mg/kg, reduced progesterone in male rats.
- CBD did not affect levels of dopamine, dopamine metabolites, or dopamine-related proteins, TH and DAT, in the striatum.
- Standard brain histopathology and immunohistochemistry are in progress.

Acknowledgments

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