U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program
Office of Chemistry and Toxicology

Examining the hepatotoxic potential of cannabidiol, cannabidiol-containing hemp extract and cannabinol at consumer-relevant exposure concentrations in primary human hepatocytes

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Publication

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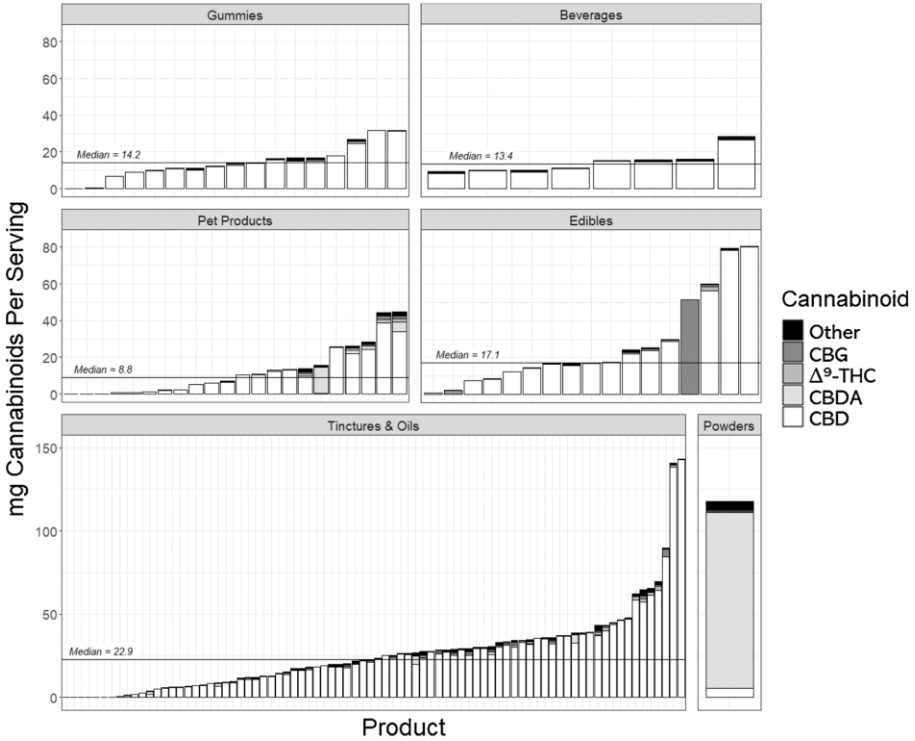
Cannabinoids and hemp-derived products on the market



Dubrow et al., 2021

- Hemp-extract
  - 120 known phytocannabinoids
    - Cannabidiol (CBD) most abundant and well studied
    - Cannabinol (CBN) first isolated cannabinoid
  - Other constituents (terpenes, toxic elements (e.g., arsenic, cadmium, mercury, and lead)
- A survey of US hemp-derived products showed a wide range of cannabinoid concentrations
  - Lowest: 0 mg/serving
  - Highest: 143 mg/serving
  - Median: 16.7 mg/serving
- Fewer than half of the products surveyed contained cannabidiol (CBD) concentrations within 20% of their label

Clinical applications of CBD can cause liver damage

CBD for treatment of seizures:

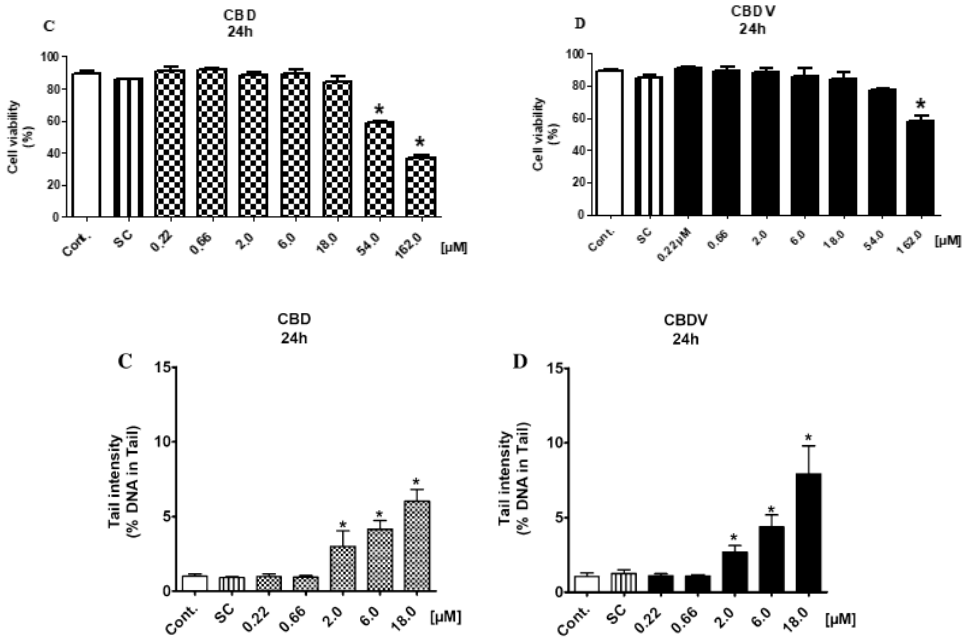
- Epidiolex: FDA- approved cannabidiol (CBD) medication to treat seizures associated with Lennox-Gastaut syndrome (LGS) and Dravet syndrome (DS)
- Concentrations administered: 1-20 mg/kg/day
- Observed C_{max}(maximum concentration of drug observed in the bloodstream after a dose is administered) : 1-3 µM (Chan 2021, Contin 2021)
- Potential Hepatotoxicity:
  - Increased liver enzymes alanine transaminase (ALT) and aspartate aminotransferase (AST) indicative of liver injury in clinical settings (especially with concomitant valproate)

Healthy adults:

- 1500 mg/day (about 20 mg/kg/day based on 70kg adult) (Watkins 2021)
- Increased liver enzymes (ALT/AST)
  - 31% of participants had elevated levels at 5 x ULN (upper limit of normal) consistent with drug-induced liver injury (DILI)
  - Large interparticipant variation

Evidence of cannabinoid-induced hepatotoxicity from in vitro cell models

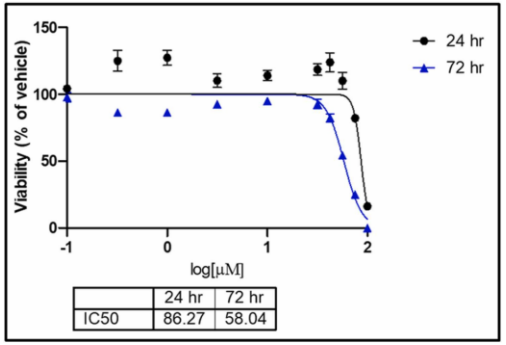
- Cannabidiol (CBD) and Cannabidivarin (CBDV) evaluation in a 2D human liver cell line (HepG2)
- Dose-dependent decrease in cell viability and increase in DNA damage after 24 hours



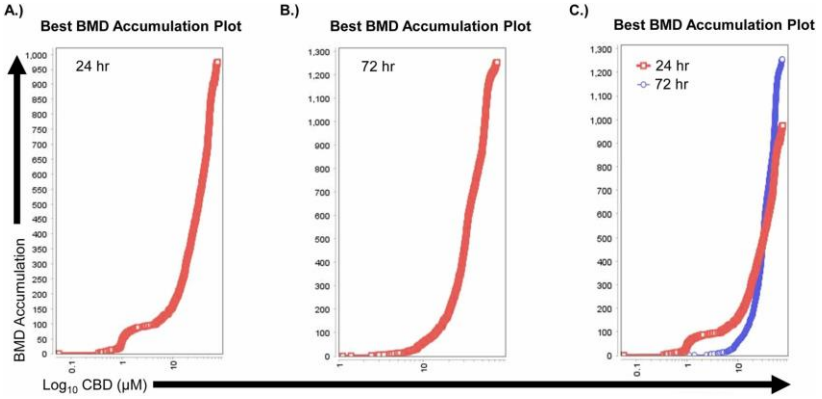
Russo et al., 2019

Evidence of cannabinoid-induced hepatotoxicity from in vitro cell models

- Cannabidiol (CBD) hepatotoxicity using human liver HepaRG cell line spheroids
- IC50 for cytotoxicity (ATP) = 86.27 µM for 24hr and 58.04 µM for 72 hr
- Human whole transcriptome TempO-Seq analysis results demonstrated an increase in the number of altered genes at greater than or equal to 10 µM



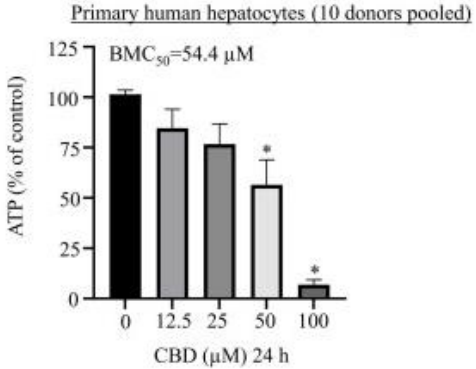
	24 hr	72 hr
IC50	86.27	58.04

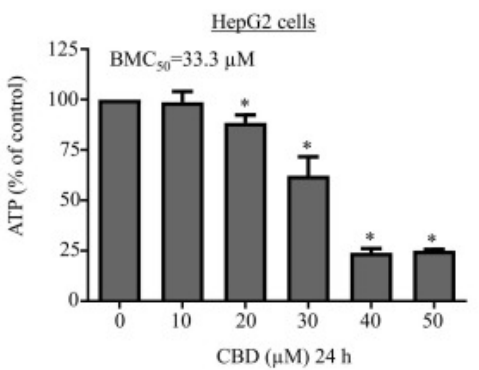


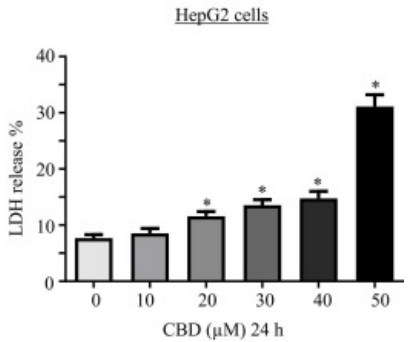
Li et al., 2023

Evidence of cannabinoid-induced hepatotoxicity from in vitro cell models

- Cannabidiol (CBD) evaluation in a 2D human liver cell line (HepG2) and primary human hepatocytes (PHH)
- Dose-dependent decrease in ATP in both PHH and HepG2, but stronger in HepG2
- Dose-dependent increase in cytotoxicity (lactate dehydrogenase, LDH) in HepG2



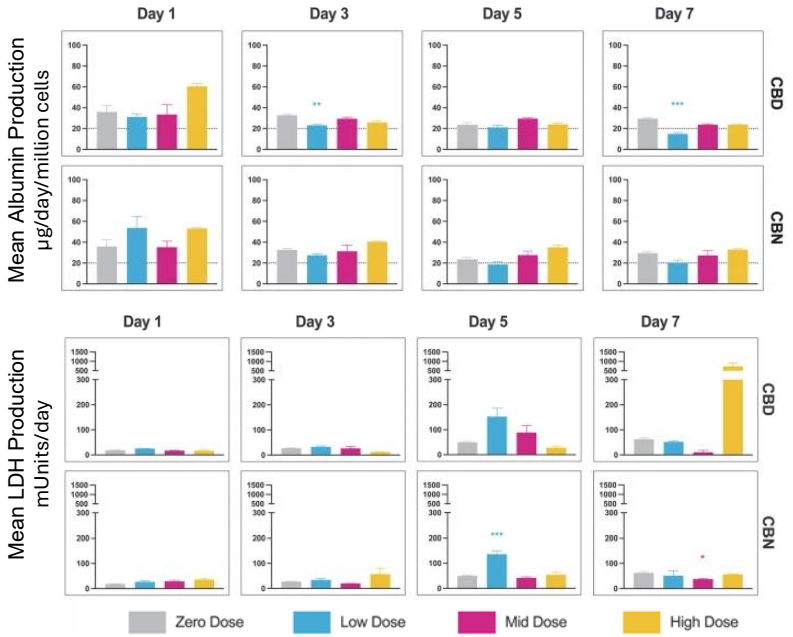




Chen et al., 2024

Evidence of cannabinoid-induced hepatotoxicity from in vitro cell models

- Cannabidiol (CBD) and Cannabinol (CBN) in quad liver-chip MPS using PHH
- Cannabinoid Concentrations:
  - 0, 0.24, 3, 4.7 µM
- Transient effects on albumin and LDH due to variability



Modified from Larson et al., 2025

Potential hepatotoxicity due to consumer-relevant exposure to hemp and its ingredients is unknown

- Step 1: In silico determination of consumer product relevant concentrations and chemical analysis of provided extracts
- Step 2: Exposure of primary human hepatocytes to chemicals and evaluation of potential hepatotoxicity

In silico modeling approach applied to determine in vitro experimental concentrations

- 30 mg (serving size) CBD oral exposure
  - Plasma C_{max}= 6 nM
  - Liver C_{max}= 86 nM
- 30 mg (serving size) CBN oral exposure
  - Plasma C_{max}= 9.1 nM
  - Liver C_{max}= 129.2 nM

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RESEARCH ARTICLE

Journal of Applied Toxicology WILEY

Physiologically based pharmacokinetic modeling and simulation of cannabinoids in human plasma and tissues

Yitong Liu | Robert L. Sprando

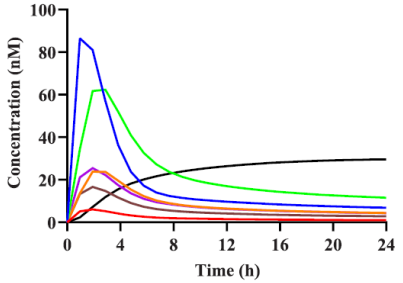
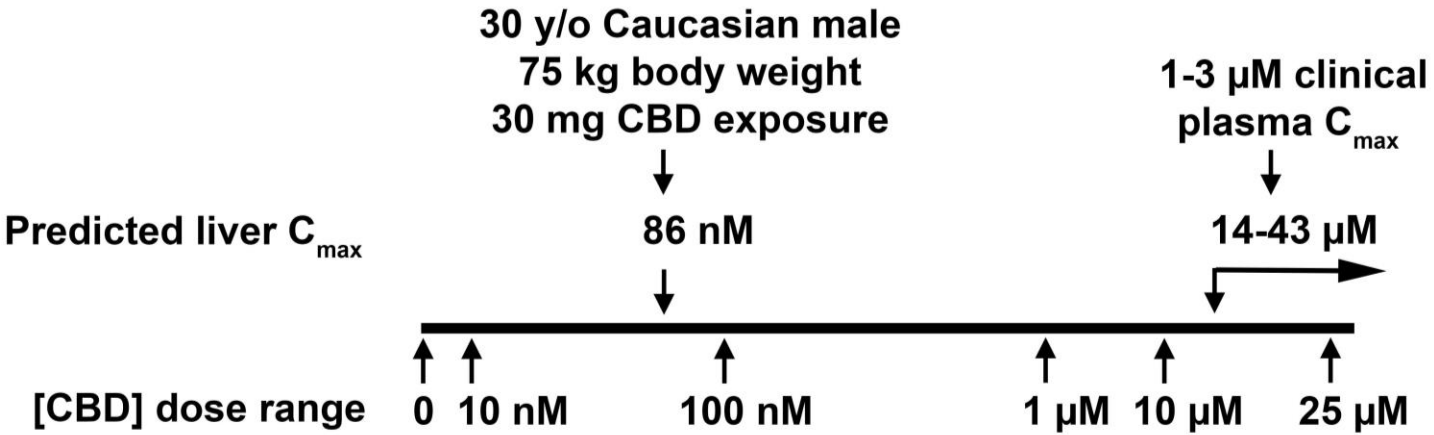


FIGURE 4 Predicted concentration and time curves of cannabidiol (CBD) in plasma and tissues after 3oral administration of 30 mg in immediate release suspension or capsule. Red curve represents plasma; blue represents liver; green represents brain; black represents adipose; purple represents kidney; orange represents reproductive organ; brown represents heart.

Prediction data applied to select an appropriate concentration-response for in vitro experiments



Striz 2024

Assumption: Plasma vs liver C_{max} relationship is linear

Chemical analysis of hemp extract

- Hemp extract received from University of Mississippi
  - Received ethanolic hemp extract; ethanol evaporated and reconstituted in DMSO for studies
- Concentration of 5 highest constituents in hemp-extract

Analyte	Measured concentration (µg/ml, mean ± SD)	Concentration (mM)
CBD	112610.56 ± 587.02	358.10
CBC	4294.45 ± 53.89	13.66
CBG	2972.59 ± 26.57	9.39
CBDV	1233.54 ± 17.39	4.31
CBN	627.53 ± 24.44	2.02

Adapted from Striz et al., 2024

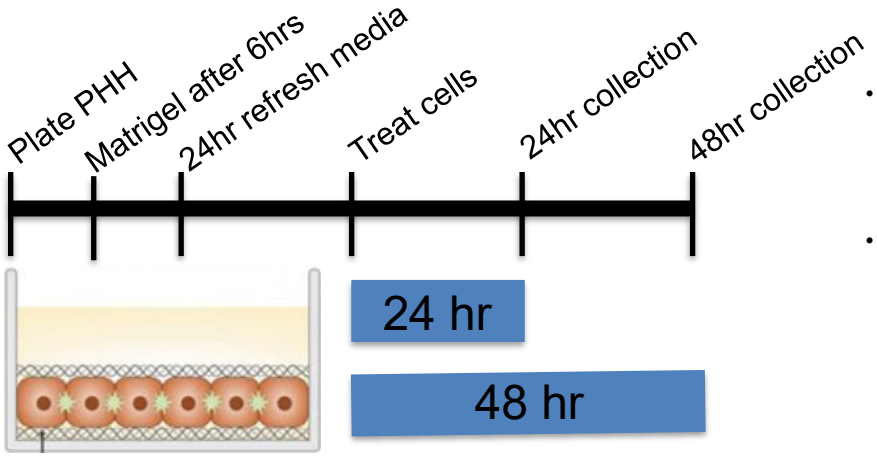
Some constituents were not stable in cell culture media

Recovery in dosing media			
Pure CBD		Pure CBN	
Conc (μM)	Avg % R	Conc (μM)	Avg % R
0.01	ND	0.01	93.20
0.1	ND	0.1	83.73
1	ND	1	75.35
10	74.14	10	75.80
25	72.68	25	74.75

Recovery (CBD) matched hemp extract cannabinoids in dosing media									
CBD		CBN		CBC		CBG		CBDV	
Conc (μM)	Avg % R	Conc (nM)	Avg % R	Conc (nM)	Avg % R	Conc (nM)	Avg % R	Conc (nM)	Avg % R
0.01	115	0.056	ND	0.38	ND	0.264	ND	0.12	ND
0.1	95.83	0.56	ND	3.8	118.42	2.64	ND	1.2	ND
1	82.47	5.6	106.25	38	104.39	26.4	96.72	12	66.81
10	85.46	56	67.26	380	87.32	264	88.93	120	60.28
25	99.52	140	72.74	950	104.16	660	107.73	300	66.78

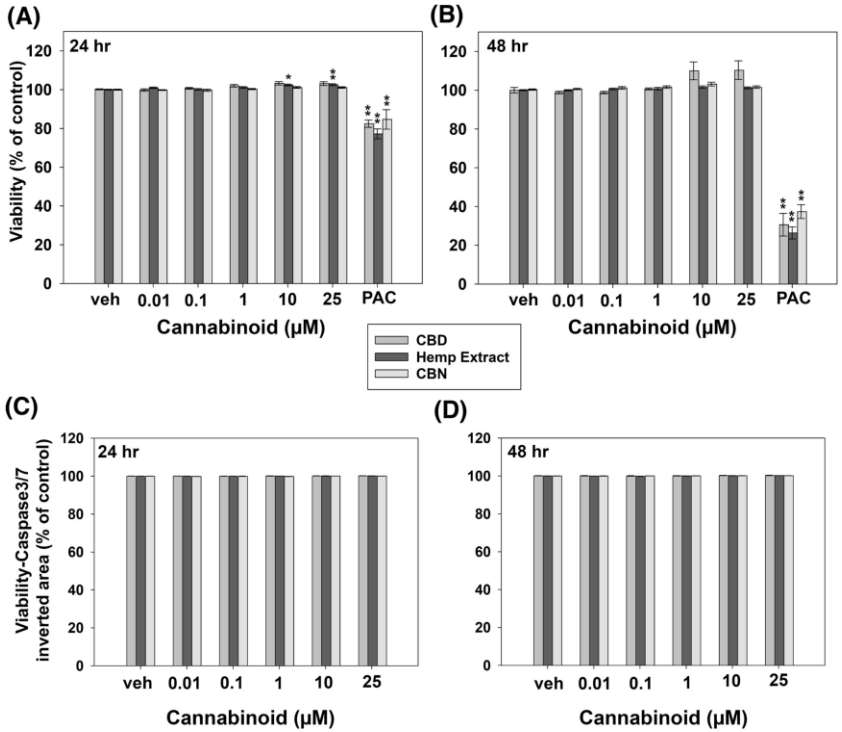
Adapted from Striz et al., 2024

Rapid toxicity study
24 & 48 hr exposure in primary human hepatocytes (PHH)



- Primary human hepatocytes (PHH)
  - 24 well plate
  - Different donors (Age 20-60)
  - 4 experiments/cannabinoid
  - 3 replicates/experiment
- Chemicals
  - CBD, CBN, (CBD)-matched Hemp extract
    - 0, 0.01, 0.1, 1, 10, 25 µM
  - Usnic acid used as positive assay control (PAC)
    - 50 µM
- Endpoints evaluated 24 and 48 hr post-exposure
  - Cytotoxicity markers
    - LDH release – cell membrane permeability
    - Caspase 3/7 activity - cell apoptosis
  - Functional Effect markers
    - Albumin secretion
    - Urea secretion
  - Mitochondrial markers
    - Tetramethylrhodamine methyl ester (TMRM) - mitochondrial membrane potential
  - Nuclei quantification
    - NucBlue

CBD, CBN, and Hemp did not elicit cytotoxicity or apoptosis

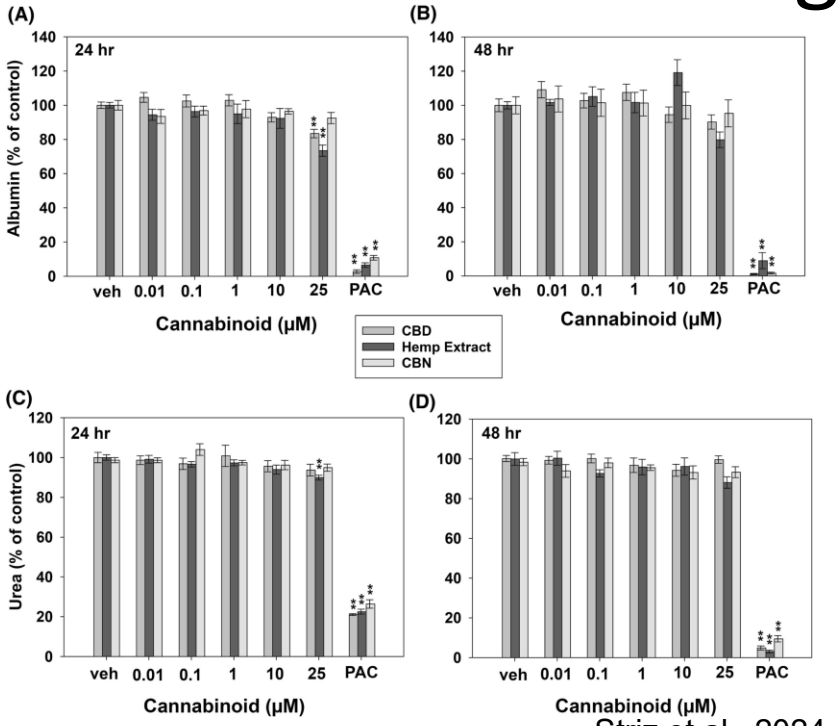


- **LDH (A&B): Cell supernatant**
  - PAC (usnic acid) significantly increased cytotoxicity
  - Cannabinoids unaltered
- **Caspase 3/7 (C&D): Imaging**
  - No change in caspase 3/7 imaging at any concentration

LDH (A&B): Results represent the inverted mean percent lysis compared to control ± SEM for four independent experiments (*)p=0.001 (**)p<0.001
Caspase 3/7(C&D): Results represent the inverted mean percent staining area compared to control ± SEM for four independent experiments.

Striz et al., 2024

Albumin and urea secretion were reduced by CBD and Hemp after 24 hr, but unchanged at 48 hr

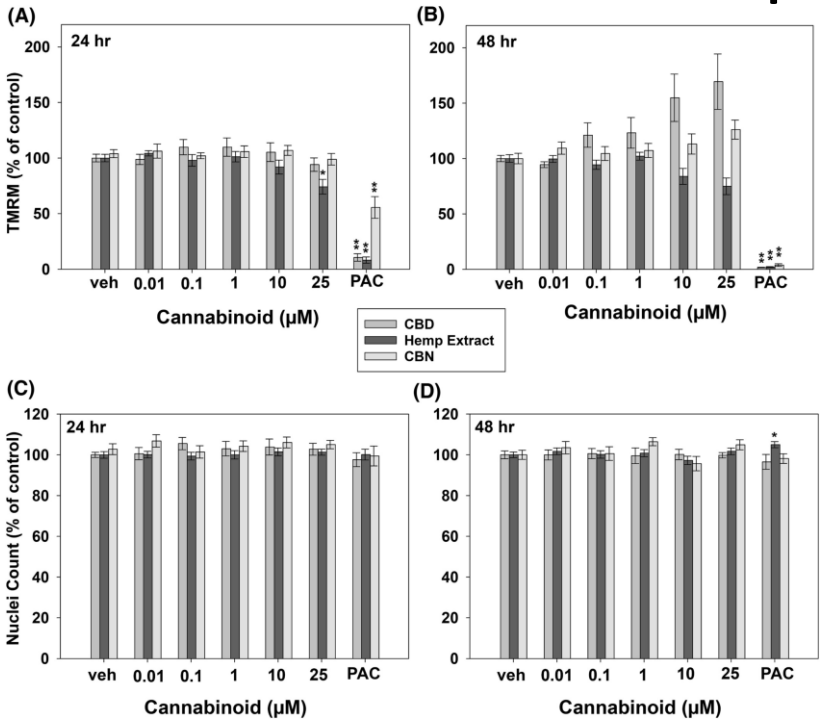


- **Albumin (A&B): Cell supernatant**
  - Reduced with 25 μM CBD (16.52%) and Hemp Extract (26.56%) from control at 24hr but not 48hr.
  - CBN not altered
- **Urea (C&D): Cell supernatant**
  - Small reduction with 25 μM Hemp Extract (10.06%) from control at 24hr but not 48hr
  - CBD and CBN not altered

Albumin (A&B): Albumin secretion was measured and plotted as percent of vehicle control. Results represent the mean ± SEM for four independent experiments. **p<0.001
Urea (C&D): Urea secretion was plotted as percentage of vehicle control. Results represent the mean ± SEM for four independent experiments. **p<0.001

Striz et al., 2024

Minimal effects on mitochondrial membrane potential observed resultant to cannabinoid exposures



Striz et al., 2024

- **TMRM: Mitochondrial Membrane Potential: Imaging**
  - CBD and CBN, observed donor variability - two donors showed increased TMRM and two did not
  - Decreased TMRM with 25 μM Hemp extract (25.85%)
- **NucBlue: Imaging**
  - No change

TMRM (A&B): The resulting staining area was divided by total area to obtain percentage coverage and then plotted as percentage of vehicle control. *$p = 0.007$, **$p < 0.001$
NucBlue (C&D) The nuclei were counted within the image area and then total well area was used to determine total nuclei/well. Nuclei count was plotted as percentage of vehicle control. Results represent 4 independent experiments.

Conclusions

- **Step 1: Chemical stability**
  - Decreased stability of cannabinoids in media could suggest that the hepatocytes did not receive the full concentration of cannabinoids presented
  - CBD was more stable in the hemp extract than in CBD alone which could explain the slightly more toxic effects of hemp extract compared to CBD alone

- **Step 2: Hepatotoxicity assessment**
- No observed cytotoxicity (LDH)
- 25 µM CBD and Hemp decreased albumin and urea
- No toxicity at less than 10 µM for any endpoint
- Inter-donor variability in TMRM

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