A Pre-clinical dose response study of a GMP-produced Cannabidiol Transdermal Formulation

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ABSTRACT

The plant *Cannabis sativa*, commonly referred to as marijuana or cannabis, has been used for its medicinal and psychotropic effects for thousands of years with records of use describing Cannabis in both Indian and Asian protocultures. There has been increasing attention recently to potential therapeutic efficacy. Many states and municipalities have legalized Cannabis in some forms when employed for medicinal purposes when used under license. Other legislative bodies in the United States, Canada, and other countries are reviewing enacting policy to facilitate freer use of cannabis or its constituents for recreational purposes. There are over 550 chemical compounds and over 100 phytocannabinoids isolated from cannabis, including cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC). THC when ingested or smoked produces psychoactive effects of cannabis, while CBD does not have similar effects. There is scientific debate as to whether CBD attenuates or exacerbates the behavioral and cognitive effects of THC, as well as the extent of the effects of CBD on THC-induced anxiety, psychosis, and cognitive deficits. Both THC and CBD, as well as other cannabinoid molecules, are currently being evaluated for inclusion in medical formularies, separately and in combination. Most notably GW Pharma has obtained an FDA registration of a CBD extract taken orally to treat a juvenile seizure disorder.

INTRODUCTION

Cannabis has been used for thousands of years for medicinal and recreational purposes. [1] Cannabidiol or CBD has become relatively available in the United States after passage of the Farm Bill in 2019 legalized the possession and sale of Cannabidiol derived from Hemp[2]. Most of the products available commercially are tinctures or extracts of hemp processing. For the most part there is no real effort made to refine or purify the active ingredients and impurities from the products and the only available dosing forms are inhaling smoke or ingesting a suspension. Smoking via a vaping system has gained some acceptance. Ingestion of the suspensions, the most common dose form, results in extremely low (4 to 6%) bioavailability as well as exposure to impurities including psychotropically active Cannabis fractions such as Delta 9 THC, so it is believed that a product which was produced to cGMP standards with an active ingredient of hemp-derived pharmaceutical purity would be attractive. There are so many sources of CBD commercially available, virtually none of which are produced to any standard approaching Good Manufacturing Practice by any Federally or state-registered facility, there is virtually no oversight of product quality.

HypoSpray Pharma has developed a proprietary system for the delivery of pharmaceutical, cosmetic, and nutritional compounds transdermally via a metered pump spray measuring a uniform 0.2 mLs of a vehicle solution composed of ethanol, fatty alcohols and food excipients and supplement actives.

A California university-based contract research organization was engaged to perform a dose response and pharmacokinetic evaluation of 3 doses of pure Hemp-derived Cannabidiol obtained from a commercial

source and formulated by The Langford Research Institute in Palm Beach Gardens, Florida, U.S.A. into its delivery system

NOTE REGARDING PRECLINICAL STUDIES

Before discussing the relevant data from this pre-clinical research on CBD effects on various parameters, several important differences between route of administration and pharmacokinetics between human and animal studies should be mentioned. Firstly, CBD has been studied in humans using oral administration or inhalation. Administration in rodents often occurs either via intraperitoneal injection or via the oral route. Second, plasma levels reached via oral administration in rodents and humans can differ. Both these observations can lead to differing active blood concentrations of CBD[3].

STUDY DESIGN

The study was designed as an open label dose range/response study comparing the serum levels achieved via the transdermal route of administration with historic data from other modes employed to deliver CBD in the Racine model.

ETHICS OVERSIGHT

The protocol was submitted to the University's Animal Care and Use Committee and the study approved.

ACTIVE INGREDIENT TESTED

Cannabidiol HDS, 5, 15 and 30 mgs Supplied by Langford Research Institute

ANIMAL HUSBANDRY

Animals Male Sprague Dawley Rats (180-220g +/- 10g) were used throughout.

Conditions: Computer-monitored Vivarium conditions to current AAALAC standards. Climate controlled with 20 air exchanges per hour and constantly monitored environment with temperature 21°C +/- 2°C, relative humidity 45-50%. Cage rooms illuminated by fluorescent light on a 12-hour light/dark cycle.

Diet: Pelleted mouse and rat No. 1 maintenance diet *ad libitum*.

Water: Mains tap water *ad libitum*.

Housing: Groups of 5 in polypropylene 4 modular cages (590x385x200mm) with animal bedding grade 7 of graded cellulose wood fibers.

Miscellaneous: Nair Hair removal Crème- contains Thioglycolate, Saline Solution

METHODS

Preparation of the rat skin: Young mature Sprague-Dawley Animals (190-220 Grams) were randomly assigned to cohorts receiving 5, 15 & 30 mg doses. Animals were received into the vivarium and allowed to acclimatize. Animals were lightly anesthetized with Halothane. A small patch of fur over the dorsum

was shaved followed by application of the depilatory crème and after 5 minutes the crème was removed using a dampened swab. The area of "nude" skin was then thoroughly cleaned with cold water and dried gently patting with a dry swab. The animals were returned to housing to recover for 48 hours.

Application of the CBD: On the test days the animals were anesthetized by means of a commercial veterinary anesthesia product containing Thiopentone Sodium USP administered intraperitoneally and were unconscious throughout the experiment so as to avoid ingesting CBD remaining on the skin via grooming behavior. Using a 1 mL disposable plastic syringe, 0.4 mLs of test material was slowly applied to the prepared rat skin, simultaneously using the index finger of a gloved hand, the test material was gently massaged onto the skin. More specifically, the index finger was rotated first clockwise 10 times then counterclockwise 10 times. 2 Hours after dosing the area was wiped clean with a wet swab then a dry swab.

Collection of samples: Serial blood draws were taken for sampling at 15 mins, 30 mins, 1 Hour, 2 Hours, 4 Hours, 8 Hours, and 24 Hours. The animals were euthanized with the final draw at 24 hours.

RESULTS

Figure 1 shows the response from serum samples in Nanograms per mL Spun Serum measured by HPLC.

Figure 1

TIME, HR→	0.25	0.50	1.00	2.00	4.00	8.00	24.00
RAT ID↓							
DOSE 5 MG							
1	21.30	65.70	125.00	170.00	103.00	34.60	22.00
2	48.50	286.00	157.00	178.00	63.60	94.60	23.50
3	3.51	234.00	86.00	75.20	60.60	21.80	16.60
4	39.20	281.00	74.60	153.00	201.00	105.00	23.70
5	8.80	41.30	224.00	215.00	125.00	61.90	16.80
DOSE 15 MG							
6	139.00	175.00	255.00	215.00	324.00	116.00	29.30
7	33.70	134.00	355.00	145.00	376.00	101.00	73.00
8	22.20	440.00	164.00	505.00	367.00	180.00	46.70
9	27.70	120.00	239.00	460.00	461.00	696.00	91.30
10	186.00	80.20	349.00	263.00	137.00	113.00	71.50
DOSE 30 MG							
11	85.20	76.30	251.00	971.00	488.00	436.00	197.00
12	576.00	1280.00	145.00	365.00	610.00	218.00	238.00
13	198.00	142.00	1630.00	475.00	1000.00	1170.00	140.00
14	688.00	126.00	121.00	322.00	599.00	737.00	147.00
15	101.00	163.00	99.60	371.00	351.00	718.00	126.00

Figure 2 shows the average values at each time point for all animals by dose .

Figure 2

TIME, HR→	Column1	Column2	Column3	Column4	Column5	Column6	Column7
DOSE 5 MG AVG	**0.25**	**0.50**	**1.00**	**2.00**	**4.00**	**8.00**	**24.00**
	24.26	181.60	133.32	158.24	110.64	63.58	20.52
TIME, HR→	**0.25**	**0.50**	**1.00**	**2.00**	**4.00**	**8.00**	**24.00**
DOSE 15 MG	81.72	189.84	272.40	317.60	333.00	241.20	62.36
TIME, HR→	**0.25**	**0.50**	**1.00**	**2.00**	**4.00**	**8.00**	**24.00**
DOSE 30 MG	82.30	170.92	268.83	426.50	358.83	273.67	84.80
Figure 6	24.26	181.60	133.32	158.24	110.64	63.58	20.52
	81.72	189.84	272.40	317.60	333.00	241.20	62.36
	82.30	170.92	268.83	426.50	358.83	273.67	84.80

Figures 3, 4 & 5 show the individual pharmacokinetic curves over the 24-hour period from dose to euthanasia.

Figure 3

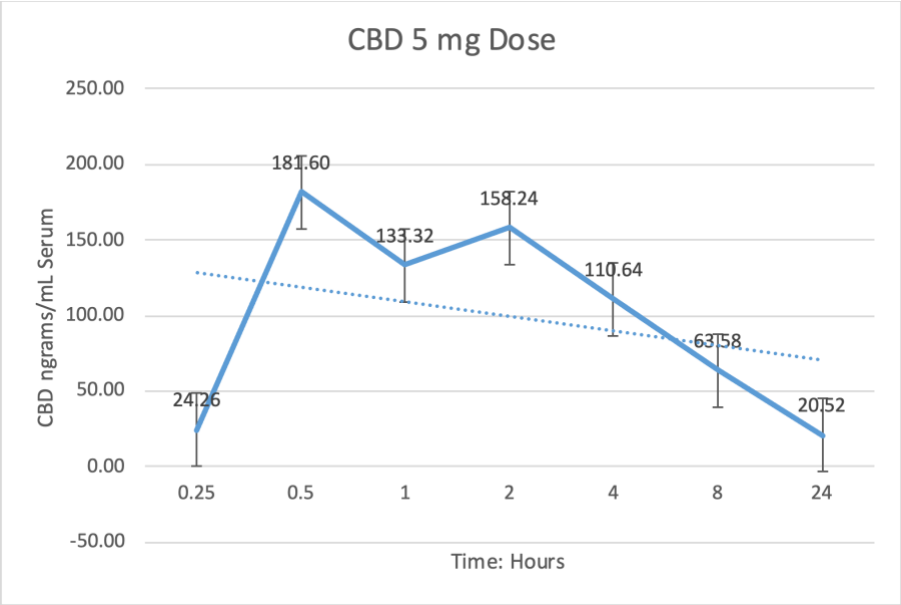


Figure 4

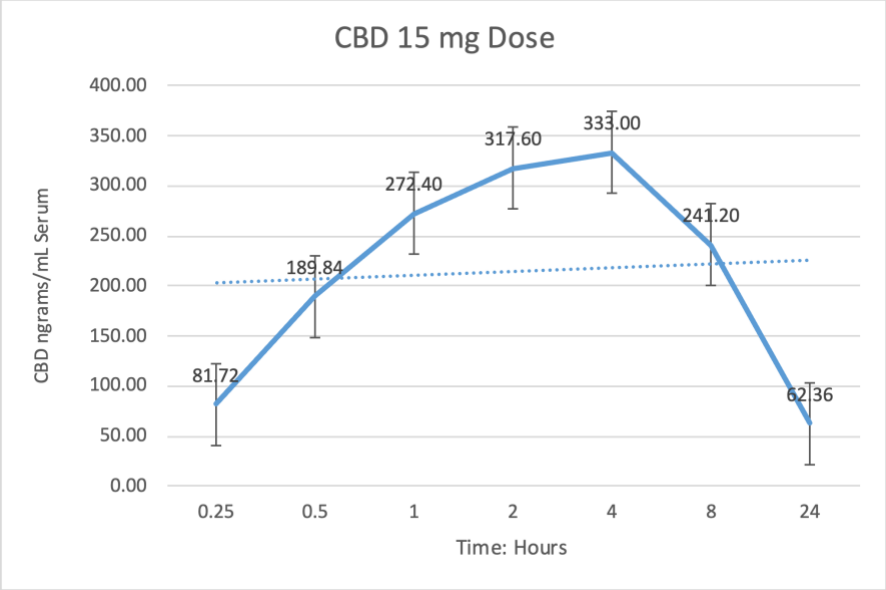


Figure 5

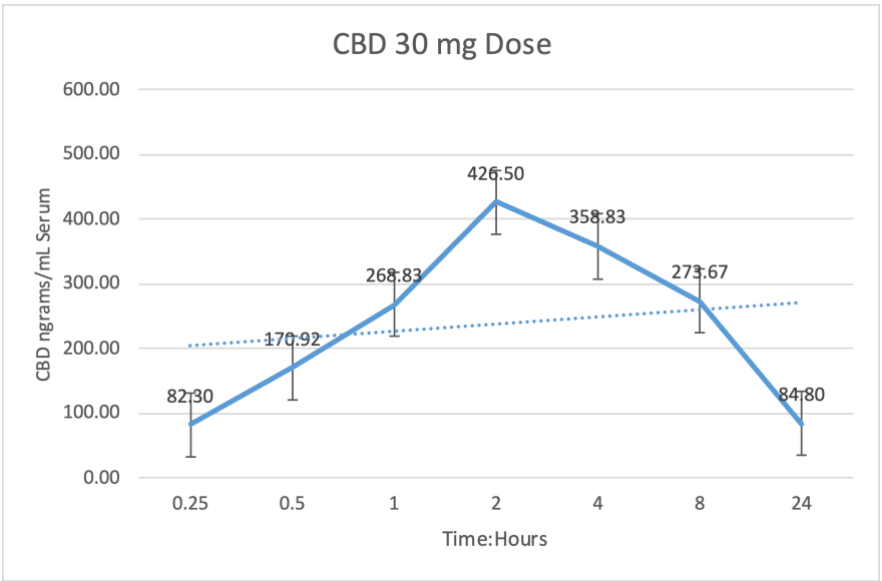
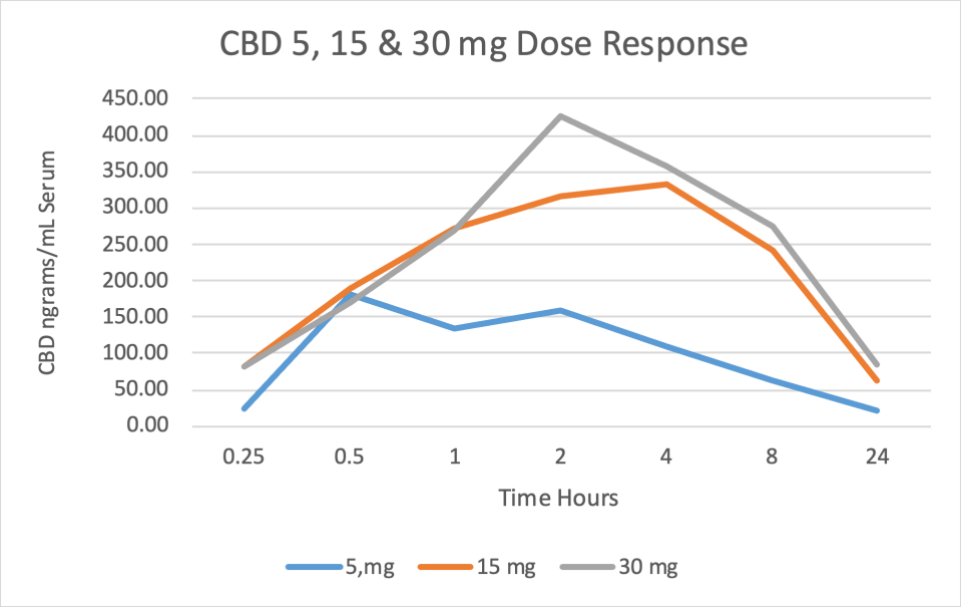


Figure 6 shows all three doses superimposed.

Figure 6



DISCUSSION

While the significant N is likely too small to draw any definitive conclusions, a few observations bear reviewing.

The transdermal dosing clearly delivered the active ingredient into the blood compartment of the animals in a dose dependent fashion. While the smallest dose (5 mg) produced a time to maximum plasma concentration (T^{MAX}) of 30 minutes versus 4 and 2 hours for the 15 and 30 mg doses respectively, Animal 3 had a variant response which affected the averages and deletion of his data yields a T^{MAX} at 2 hours. Even with Animal 3's data included, the 5 mg dose response is well within the accepted therapeutic level at 30 minutes. In fact, all doses show a response greater than 20 ng/dL at 30 minutes with 5 mg equating to 340 mg Dose in humans. Figure 7 shows the Mouse and Rat to Human CBD Dose Conversion.

Figure 7

Mouse to Human CBD Dose Conversion		Rat to Human CBD Dose Conversion	
Mouse Dose ($mg \cdot kg^{-1}$, i.p.)	HED (mg, p.o.)	Rat Dose ($mg \cdot kg^{-1}$, i.p.)	HED (mg, p.o.)
1	34	1	68
5	170	5	340
10	341	10	681
20	681	20	1362
30	1021	30	2043
60	2043	60	4086

Each HED is based on a body mass of 60 kg and calculated as per the methods described in 2.3 *Dose Conversions*. The highest documented acute oral CBD dose in humans is 6000 mg; the highest documented chronic oral CBD dose in humans is 1500 mg [169]. HED: Human Equivalent Dose; i.p.: Intraperitoneal; p.o.: Oral

In all 3 doses all animals returned to pre-dose endogenous levels of CBD at 24 hours post dose.

SUMMARY

Again, while a cohort N of 5 is low, the response data is suggestive of a good and predictable pK profile clearing in 24 hours with the transdermal dose form that is worthy of further study.

THANKS

We want to offer particular thanks to Dr. Jeremiah Momper, and Arnold Garcia, MsC. And their analytics team at UC San Diego for their invaluable assistance with this project.

[1] Callaway J (2004). Hempseed as a nutritional resource: an overview. Euphytica 140: 65–72.
2 Abernethy A, MD, PhD., Principal Deputy Commissioner - Office of the Commissioner, (2019) https://www.fda.gov/news-events/congressional-testimony/hemp-production-and-2018-farm-bill-07252019

3 Iffland K; Grotenhermen F; An Update on Safety and Side Effects of Cannabidiol: A Review of Clinical Data and Relevant Animal Studies; Published Online:1 Jun 2017https://doi.org/10.1089/can.2016.0034