Characterization of Cannabis Smoke Condensate and its Inhibitory Effects on CYPs in Human Lung

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BACKGROUND

Smoked cannabis flower is the primary form of medical cannabis (MC, Figure 1) recommended for various conditions in Florida and elsewhere, and it's also widely used recreationally (1). MC is commonly utilized to manage chronic ailments like amyotrophic lateral sclerosis, cancer, Crohn's disease, epilepsy, and glaucoma, among others. The growing use of MC, including in lung diseases, raises concerns about potential drug-drug interactions (DDIs).

The major bioactive compounds in MC are cannabinoids (CBs) including Δ-9-tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) (2). Additionally, a significant but bio-inactive CB is cannabigerol (CBG), which serves as the basic structural compound in the biosynthesis of other CBs in the cannabis plant (2). Various research reports have highlighted potential inhibitory DDIs between major cannabinoids. In vitro studies have shown that THC, CBD, and CBN can potently inhibit several drug-metabolizing enzymes (DMEs), including cytochrome P450 (CYP450) and carboxylesterase 1 (CES1) (3).

Figure 1. Cannabis plant

The lung is recognized as a primary organ for drug metabolism, with several DMEs expressed within the lung (4). However, limited studies have explored the potential DDI of MC through the inhibition of lung DMEs. This study aims to collect and characterize cannabis smoke condensate (CSC) and assess its in vitro inhibitory effects on prominent DMEs in the lung.

METHOD

Three standardized cannabis cigarettes sourced from the US National Institute of Drug Abuse (NIDA) Drug Supply Program (5) were consecutively combusted in an enclosed smoke exposure system (Figure 2). Generated smoke was routed through an ultra-cold condenser permitting the collection of cannabis smoke condensate. The condensate was weighed and analyzed for the presence of 8 major CBs via LC-MS/MS. In vitro enzyme inhibition studies were conducted/ongoing using human lung S9 to evaluate the potential inhibitory effect of cannabis smoke condensate on the lung.

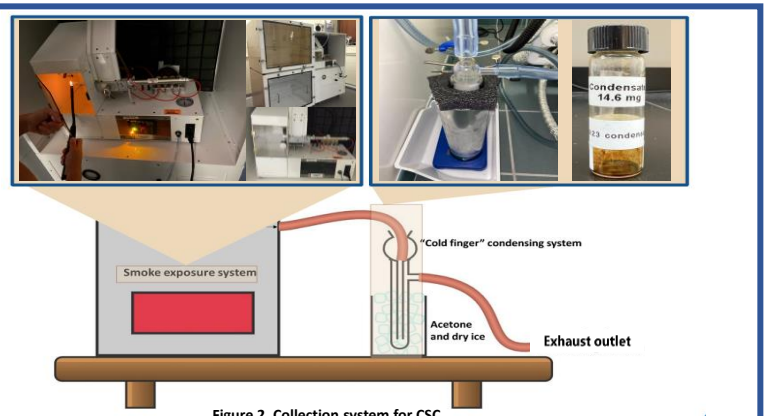


Figure 2. Collection system for CSC.

Figure 3. CSC in the condenser (left), final CSC concentrate (middle), and cannabis plant extraction (right).

Table 1. Characterizing DME expression in lung and liver tissues

Enzymes	Lung	Liver	Examples of Substrates
CYP450s			
CYP1A2	+/-	+++	Caffeine, theophylline, tizanidine, ramelteon, endogenous melatonin
CYP1B1	++	+	Melatonin, estrogens, retinoic acid
CYP2A6	++	+++	Cotinine, efavirenz, valproic acid, pilocarpine, caffeine
CYP2B6/7	+++	+++	Cyclophosphamide, ifosfamide, propofol, ketamine, methadone, nevirapine, efavirenz
CYP2C8	+/-	+++	Amodiaquine, cerivastatin, dasabuvir, enzalutamide, imatinib, loperamide, montelukast, paclitaxel, pioglitazone, repaglinide, rosiglitazone
CYP2C9	+/-	+++	Fluoxetine, losartan, phenytoin, tolbutamide, torsemide, S-warfarin
CYP2C19	+/-	+++	Warfarin, diazepam, omeprazole lansoprazole, pantoprazole, imipramine, amitriptyline, clomipramine, citalopram phenytoin, mephenytoin, phenobarbitone, hexobarbital, teniposide, cyclophosphamide, propranolol, indomethacin
CYP2D6	+/-	+++	Codeine, tramadol paroxetine, tricyclic antidepressants, metoprolol, bisoprolol, tamoxifen
CYP2E1	+++	+++	Halothane, enflurane, isoflurane, paracetamol, ethanol, theophylline, chlorzoxazone, zopiclone, ezopiclone and verapamil
CYP2F1	+++	-	Naphthalene, ethoxy coumarin
CYP3A4	+/-	+++	Midazolam, acetaminophen, codeine, ciclosporin, diazepam, erythromycin, and chloroquine
CYP3A5	+++	+++	Tacrolimus, sirolimus, cyclosporine
ESTERASES			
CES1	++	+++	Methylphenidate, lovastatin enalapril, cocaine, clofibrate, quinapril, nifinamide, fenofibrate, heroin, ramipril, meperidine, oseltamivir, remdesivir
PON2	+++	+++	Endogenous oxidative

PRELIMINARY RESULTS

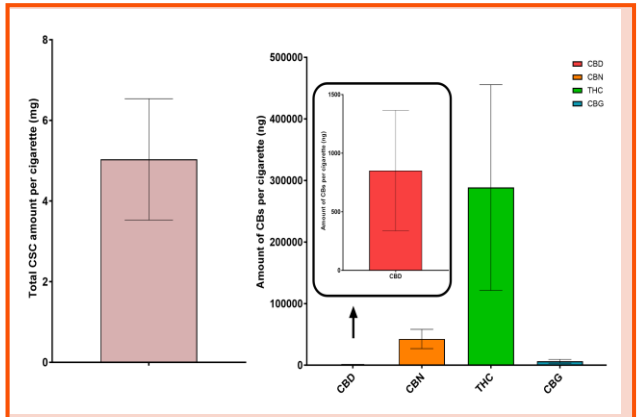
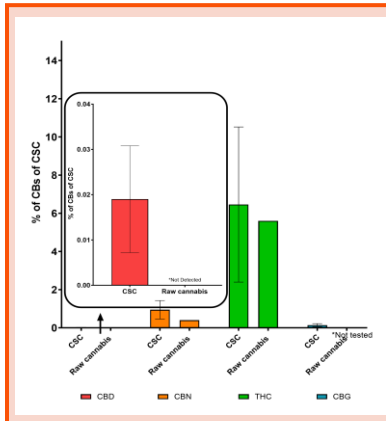


Figure 4. Amount of CSC and individual CBs from collections (n=3).
Left: Total amount of CSC. *Right*: Amount of each cannabinoid



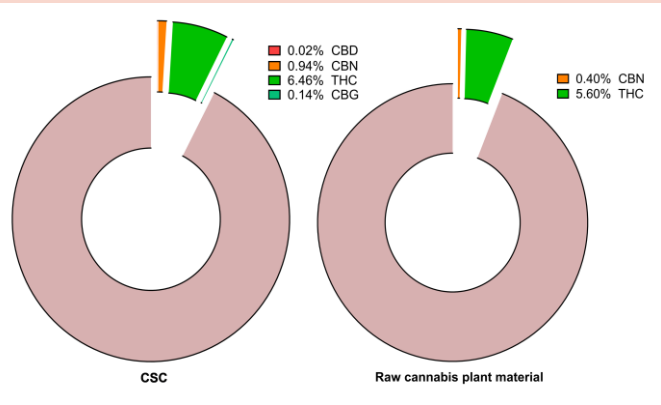


Figure 5. Percentage of major cannabinoids relative to the total amount of CSC or raw cannabis plant material.
raw cannabis plant material data sourced from the US NIDA Drug Supply Program (5).

Table 2. Amount of CSC and individual cannabinoids from three separate collections

		Collection 1	Collection 2	Collection 3	Mean	SD
Number of cigarettes		2	2	3		
Amount of CSC (mg)		7.1	13.2	14.6	11.6	4.0
Amount of CSC per cigarette (mg)		3.6	6.6	4.9	5.0	1.5
Measured amount per cigarette (ng)						
	CBD	763.3	371.6	1396.7	851.8	514.1
	CBN	45440.0	25684.9	56615.6	42580.2	15662.4
	THC	292875.0	119350.0	453411.1	288545.4	167072.5
	CBG	6472.8	3302.2	9392.7	6389.2	3046.0
Percentage of CBs in CSC						
	CBD	0.022%	0.006%	0.029%		
	CBN	1.280%	0.389%	1.163%		
	THC	8.250%	1.808%	9.317%		
	CBG	0.182%	0.050%	0.193%		

Table 3. CYP Activities in HluM and HLS9.

CYPs	Index Reaction		Metabolite formation rate		The ratio of HluM/HLS9 (%)
			HLS9	HluM	
CYP2B6	Bupropion	OH bupropion	0.0047	0.0002	3.5%
CYP2C19	Omeprazole	5-OH Omeprazole	0.0347	0.0005	1.6%
CYP2C8	Repaglinide	3′-OH repaglinide	0.5660	0.0005	0.1%
CYP2C9	Tolbutamide	4-OH tolbutamide	0.0012	n.d.	n.d.
CYP2D6	Dextromethorphan	Dextrorphan	0.3370	0.0006	0.2%
CYP2E1	Chlorzoxazone	6-OH chlorzoxazone	0.0199	n.d.	n.d.
CYP3A4	Midazolam	α-OH midazolam	1.2400	0.0106	0.9%

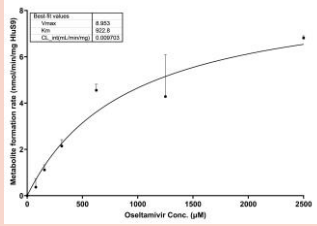


Figure 6. Activity of CES1 in human lung S9 fractions (HluS9).

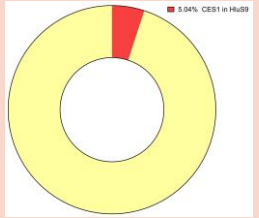


Figure 7.
Percentage of CES1 amount in HLuS9.

* Values are calculated based on the current experimental results and the transfected embryonic kidney cell line expressing CES1 incubation.

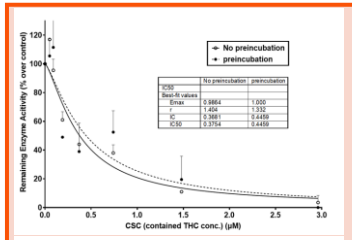


Figure 8. CSC reversibly inhibits CES1 activity with HluS9 incubation. Probe substrate: oseltamivir.

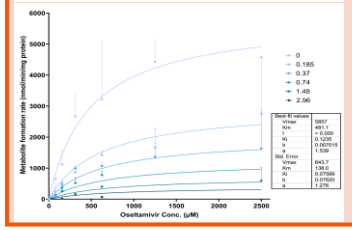


Figure 9. CSC strongly inhibits CES1 activity with HluS9 incubation. Ki = 0.12 μM.

CONCLUSION

- A total of 14.6 mg (4.87 mg per cigarette) of CSC was collected.
- CSC containing 0.022% CBD, 1.163% CBN, 9.317% THC, 0.193% CBG, and 0.008% 11-OH-THC of the total weight.
- The study revealed that the relative CB content of cannabis smoke is substantially different from that of un-combusted cannabis plant material.
- The half-maximal inhibitory concentration (IC50) of CSC for CYP3A inhibition was determined to be 17.85 μM with THC as the index substrate.
- To date, seven tested CYPs have exhibited less than 5% activity in HluM compared to HluS9.
- CSC produced a mild inhibitory effect on CYP3A activity and a strong inhibitory effect on CES1 activity.
- Continued investigations are ongoing to examine additional enzyme activities with high mRNA levels in the lung, including CYP2F1 and CYP3A5. Further investigations are ongoing to elucidate the inhibition mechanisms and assess the inhibitory effects on other CYPs

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6. Cannabis illustration by PharmD candidate Kelly Ho.

ACKNOWLEDGEMENTS

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Consortium for Medical Marijuana Clinical Outcomes Research