An *in vitro* evaluation of mitragynine metabolism to 7-hydroxymitragynine and 9-O-demethylmitragynine and the influence of major cannabinoids

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INTRODUCTION

Kratom (*Mitragyna speicosa* Korth.) is an indigenous tree native to Southeast Asia, whose leaves have been traditionally ingested in raw form by chewing or drinking as a tea[1,2]. In recent years, kratom's popularity has risen in the U.S. with an estimated usage prevalence of 1% (~ approximately 3 million people)[3]. Although kratom and its constituents presently have no FDA approved uses, it's extracts have psychoactive properties that may hold promise for the treatment of opioid cessation and pain management – in part due to its ability to partially agonize mu opioid receptors[4]. Kratom's pharmacological effects are attributed to its major indole alkaloids, such as **mitragynine (MTG)** and **7-hydroxymitragynine (7HM)**[4]. While kratom extracts contain over dozens of different alkaloids, MTG is the most abundant, comprising well over 60% of alkaloid content[4].

Cannabis [*Cannabis sativa* L. (marijuana)] has seen a dramatic increase in legal access and medicinal use in the State of Florida and the U.S for numerous indications (i.e., chronic pain, nausea and vomiting)[5]. Cannabis contains several psychotropic active compounds, but the major cannabinoids: cannabidiol (CBD), ∆-9-tetrahydrocannabinol (THC), and cannabinol (CBN) have been frequently cited for their therapeutic effects[6]. Both entities are widely available, many cannabis dispensaries also sell kratom and *combination products* containing CBD and kratom, so concurrent use is likely, and with it, the risk of potential botanical-drug interactions.

Recently, there has been a widening interest on the potential for both cannabis and kratom to potentially participate in drug interactions by inhibiting various drug metabolizing enzymes (DMEs) as well as investigating the DMEs responsible for the formation of major metabolites. The cytochrome P450 (CYPs) is a prominent family of enzymes found primarily in the liver that are partially responsible for the metabolism of most pharmaceutical agents.

CBD, THC, and CBN have demonstrated inhibition of the major CYPs (i.e., 3A4, 2D6, etc.) to varying degrees[7]. Although 7HM exists in trace amounts in kratom extracts, it has been primarily appreciated as the major active metabolite of MTG formed via oxidative metabolism catalyzed by CYP3A[8,9]. However, **9-O-demethylmitragynine (9ODM)** has also been reported as the most abundant active metabolite of MTG formed via O-demethylation mediated by CYPs 2C19, 2D6, and 3A4[9,10]. **As both metabolites are considered active, it is important to delineate possible metabolic pathways responsible for their formation since genetic polymorphisms and drug-drug interactions (DDIs) can potentially influence the safety and toxicity profile of kratom formulations, especially in the context of concomitant usage with cannabis products.**

OBJECTIVES

1. To assess the metabolic pathways of kratom's major alkaloid (>60%), MTG and major in vitro metabolites formed.
2. To determine the in vitro metabolic parameters and intrinsic clearance of MTG through CYP enzymes.
3. To assess the DDI risk when concomitantly ingesting kratom products and cannabis-containing products containing one or more cannabinoid (i.e., CBD, THC, and CBN).

METHODS

- An *in vitro* incubation system comprised of a phosphate buffer system (100 mM, pH 7.4), human liver S9 (HLS9) purchased from XenoTech (Kansas City, KS), 5 mM MgCl, 0.05 mM $NADP^+$, 3.75 mM DL-isocitric acid, and 1 unit/mL isocitric dehydrogenase, MTG at varying concentrations, and 5% DMSO was employed to represent CYP metabolism. The formation of 7HM and 9ODM were measured by LC-MS/MS analysis.
- Metabolic formation from substrate, MTG, were determined by using the non-linear curve fitting: Michaelis-Menten enzyme kinetics [Eq.1]:

$$V = \frac{V_{max}[S]}{K_m + [S]} \quad \text{[Eq.1]}$$

where V represents product formation, V_{max} represents the maximum velocity of reaction, [S] represents substrate concentration of MTG, or subs, and K_m represents the Michaelis-Menten constant.

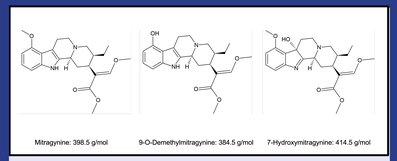


Figure 1. Chemical structures and molecular weights of select kratom alkaloids and major metabolites

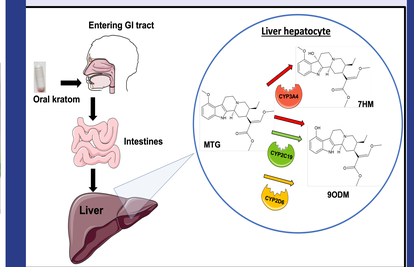


Figure 2. CYP mediated metabolism of kratom's major alkaloid, MTG, to 7HM and 9ODM.

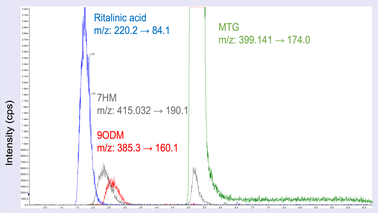
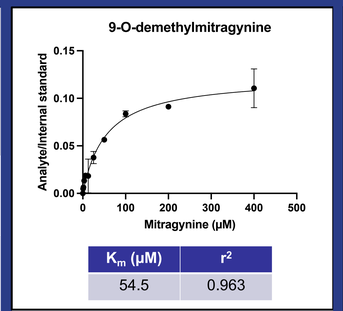


Figure 3. Chromatogram of internal standard ritalinic acid, substrate MTG, metabolite 7HM and metabolite 9ODM.

RESULTS



K_m (μM)	r^2
54.5	0.963

Figure 4. Kinetic analysis of MTG metabolism mediated through CYPs.

HLS9 (0.25 mg/mL) were incubated for 60 min with varying concentrations of MTG. Data points represent mean (±SD) of duplicate samples. The line represents the nonlinear regression analysis from [Eq.1]. Parameter estimates were determined using [Eq.1].

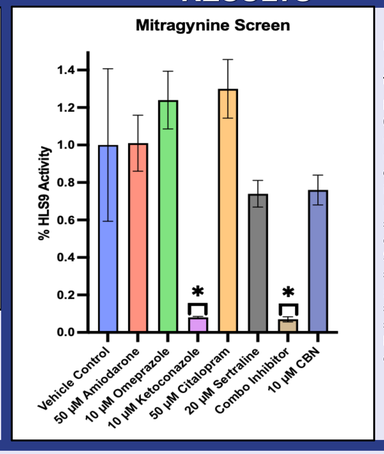
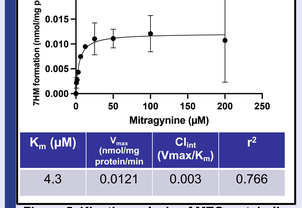


Figure 7. Inhibition screen for known CYP inhibitors and CBN on formation of 9ODM from MTG.

HLS9 (0.25 mg/mL) were incubated for 60 min with 50 μM of MTG and respective inhibitors. Each bar represents the velocity of the formation of 9ODM relative to control with no inhibitor (Dashed line N.C.). The bars represent the mean (±SD) of triplicate samples. The combination inhibitor contains a total of 10 μM omeprazole, 5 μM ketoconazole, and 20 μM sertraline. A one-tailed test was performed to determine statistical significance (α = 0.05). (*) indicates statistical significance ($p < 0.05$) when HLS9 activity was reduced below control.

Table 1. List of known inhibitors for the CYP enzymes.

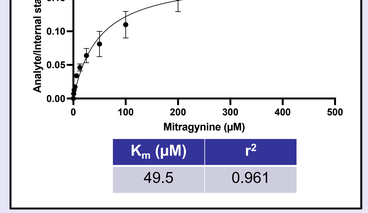
Compound	CYP2C19 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor
Amiodarone	None	**Weak**	None
Omeprazole	**Moderate**	None	None
Ketoconazole	**Moderate**	None	**Strong**
Citalopram	**Weak**	**Weak**	None
Sertraline	None	**Weak**	None



K_m (μM)	V_{max} (nmol/mg protein/min	Cl_{int} (V_{max}/K_m)	r^2
4.3	0.0121	0.003	0.766

Figure 5. Kinetic analysis of MTG metabolism mediated through CYPs.

HLS9 (0.42 mg/mL) were incubated for 60 min with varying concentrations of MTG. Data points represent mean (±SD) of duplicate samples. The line represents the nonlinear regression analysis from [Eq.1]. Parameter estimates were determined using [Eq.1]. 400 μM was excluded due to insufficient formation of 7HM.



K_m (μM)	r^2
49.5	0.961

Figure 6. Kinetic analysis of MTG metabolism mediated through CYPs.

HLS9 (0.42 mg/mL) were incubated for 60 min with varying concentrations of MTG. Data points represent mean (±SD) of duplicate samples. The line represents the nonlinear regression analysis from [Eq.1]. Parameter estimates were determined using [Eq.1].

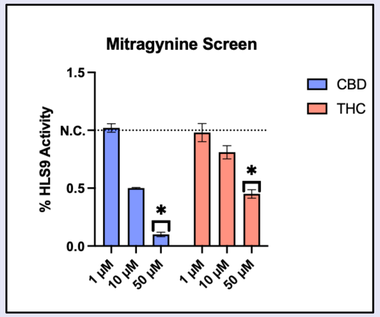


Figure 8. Inhibition screen for CBD and THC on formation of 9ODM from MTG.

HLS9 (0.25 mg/mL) were incubated for 60 min with 50 μM of MTG and respective inhibitor. Each bar represents the velocity of formation of 9ODM relative to control with no inhibitor (Dashed line N.C.). The bars represent the mean (±SD) of triplicate samples.. A one-tailed test was performed to determine statistical significance (α = 0.05). (*) indicates statistical significance ($p < 0.05$) when HLS9 activity was reduced below control.

CONCLUSIONS

- 9ODM was observed as the major *in vitro* metabolite for MTG metabolism, while limited 7HM formation was observed.
- The K_m (Michaelis-Menten Constant) for 9ODM formation through HLS9 was approximated at 50 μM.
- Ketoconazole, a strong inhibitor of CYP3A4, significantly inhibited the formation of 9ODM, whereas other inhibitors of CYP2C19 and 2D6 did not show a significant inhibition of formation of 9ODM.
- CBN inhibited 9ODM formation by 24%, and CBD and THC concentration dependent inhibition on 9ODM formation. 50 μM for CBD (90%) and THC (55%) were the only concentrations that showed significant inhibition of 9ODM formation.
- **CBD more potently inhibits 9ODM formation than THC, due to CBD being a more potent inhibitor of CYP3A4 and 2D6.**

FUTURE DIRECTIONS

- Tune and develop LC method and standard curve for 9ODM to accurately determine 9ODM formation rate.
- Quantify the *in vitro* kinetic parameters for 9ODM formation and determine intrinsic clearance of MTG to 9ODM.
- Quantify the contribution of CYP2C19, 2D6, and 3A4 metabolism from MTG to 9ODM.
- Determine and quantify the impact (inhibition constant or Ki) of known CYP inhibitors as well major cannabinoids on metabolism of MTG to 9ODM.

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