Postmortem Mitragynine Distribution in a Single Drug Fatality Case

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ABSTRACT

A 32-year-old Caucasian male was found unconscious at his sober-living home and pronounced dead after transportation to the emergency room. The decedent had a documented history of substance-use disorder and past suicide attempts, but according to his family, he was sober for the past year. Significant autopsy findings were cardiomegaly, hepatomegaly, congested lungs, cerebral edema, and obesity. The toxicology examination of blood and tissues using liquid chromatography tandem mass spectrometry detected only mitragynine in the central blood (7.5 mg/L), peripheral blood (3.3 mg/L), liver (42.2 mg/kg), and gastric contents (33.1 mg). The qualitative identification of 7-hydroxymitragynine was performed only on the central blood. The pathologist ruled the cause of death acute mitragynine intoxication combined with cardiomegaly with left ventricular hypertrophy, with severe hepatomegaly and obesity listed as other significant conditions. The mode, or manner, of death was determined to be an accidental overdose. To the authors' knowledge, this is the first reported case where mitragynine was the only drug detected. This case study will contribute to the understanding of mitragynine-only death investigation and provide valuable toxicology information for medical examiners and pathologists.

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INTRODUCTION

Mitragynine is the primary active alkaloid from the plant *Mitragyna speciosa*, a tree commonly found in Southeast Asia (1-3). The plant contains several active alkaloids, with mitragynine being the most abundant. Another known active constituent is 7-hydroxymitragynine, which is reported to be approximately 13-times more potent than morphine and approximately 46-times more potent than mitragynine (1).

However, mitragynine and 7-hydroxymitrgyanine are found at approximately 2% and 0.3%, respectively, by mass in *Mitragyna speciosa* (1, 3).

Also known as Kratom, mitragynine has historically been used in teas or chewed for its stimulant qualities by people in Southeast Asia (1, 3). Recently, in Western culture, it has become popular for its euphoria and increased energy effects when ingested as a tea or as a powder contained in a capsule (3). Many users and distributors of the drug also claim that Kratom can be used as a modern-day herbal alternative to opioids for pain management or even to aid in ending opioid dependency (2, 3).

Despite considerable investigation into the mechanism of action, the exact interactions in which mitragynine and 7-hydroxymitragynine behave on the μ-opioid receptors remains uncertain (3). However, the multiple activity models researched can help explain the wide range of effects seen after ingestion (3). The effects of mitragynine appear to be dose-dependent; lower doses are associated with stimulant effects and higher doses are related to opioid-like effects (2). Although mitragynine involvement in death investigation cases is complex, there are common adverse reactions observed after the ingestion of mitragynine. Side effects include tachycardia, agitation/irritability, drowsiness, nausea, hypertension, decreased appetite, anorexia, weight loss, insomnia, sweating, tremors, and constipation (1-3). Long-term use has been reported to include liver toxicity and high doses have been reported to cause seizure, coma, or both (3, 4).

Kratom use in the United States is estimated to be approximately 0.8% of adults 18 years and older (2). In Orange County, California, this percentage is consistent (5). Mitragynine is legal in California and there are numerous establishments that serve Kratom within Orange County. This paper aims to add to the knowledge of mitragynine and its involvement during medicolegal death investigation cases with a thorough history along with comprehensive toxicology and autopsy results.

METHODS AND MATERIALS

Reagents and Standards

Internal standards used for liquid chromatography-quadrupole time-of-flight (LC-QTOF) screening method, nortriptyline-d_3, oxazepam-d_3, oxycodone-d_6, and PCP-d_5, were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Instrument LC solvents of acetonitrile, formic acid, isopropanol, and methanol were purchased from Fisher Scientific (Hampton, NH). Ultra-pure water was used at 18.2 MΩ and obtained from Merck Millipore Milli-Q Direct-8 Ultrapure water system (Burlington, MA). Sodium fluoride and potassium oxalate monohydrate were purchased from MilliporeSigma (Burlington, MA). Liquid chromatography tandem mass spectrometry (LC-MS/MS) quantitation used internal standard of 1 mg/mL mitragynine-d_3 purchased from Cerilliant (Round Rock, TX). Drug standard 1 mg/mL of mitragynine purchased from Lipomed (Cambridge, MA). DPX WAX-S tips were purchased from DPX Technologies (Columbia, SC).

Sample Preparation

Cardiac blood, femoral venous blood, liver, and gastric contents were obtained after a comprehensive medicolegal autopsy from the Orange County Coroner's Department. Postmortem blood was stored at 4°C, while peripheral blood, liver, and stomach contents were stored at −20°C. Cardiac blood and femoral venous blood were stored with 2% sodium fluoride and 0.75% potassium oxalate. Liver and gastric contents were stored without preservatives.

Liquid Chromatography QTOF Qualitative Identification

Liquid chromatography mobile phases were an organic solvent (B) at 100% methanol with 0.1% formic acid and an aqueous solvent (A) at 100% ultra-pure water with 0.1% formic acid. The LC method started with a flow at 0.7 mL/min of 90% A, with linear ramp to 2% A, 98% B to 7.5 minutes, and held until 8.5 minutes. The final minute of the method reset the mobile phases to their initial conditions. The method used Phenomenex Kinetex® Phenyl-Hexyl, 2.6 µm, 50 × 4.6 mm with UHPLC Phenyl Guard Column for 4.6 mm ID columns at 40°C. Autosampler temperature was set at 10°C with injection volume of 10 µL. Instrument ion source contained a Turbo IonSpray with a curtain gas of 30 psi, Source gas 1 and 2 at 60 psi with a temperature of 600°C.

Extraction procedure started with 0.5 mL of standards, sample (blood or urine only), and blank, which were vortexed and centrifuged for 10 minutes after the addition of 1.5 mL internal standard. Internal standard consisted of 150 ng/mL of each PCP-d_5, nortriptyline-d_3, and oxazepam-d_5, and 300 ng/mL of oxycodone-d_6. Supernatant was transferred to clean tubes and dried under heated air (~40°C) before reconstitution with 0.5 mL of initial mobile phase.

Sciex QTOF data processing of samples used a workflow of targeted identification by best fit and through information dependent analysis data collection to search against the in-house library. Integration parameters included minimum peak width of 3 points, minimum peak height at 1000 response, S/N integration threshold of 3; gaussian smooth width of 1.0-point, noise percentage of 80%; retention time half window at 15 seconds. Regression parameter of area and linear through zero type without weighting. The method was validated following the ANSI-ASB Method Validation for Forensic Toxicology Standards at an LOD of 2 and 10 ng/mL for 7-hydroxymitragynine and mitragynine, respectively (6, 7). Minimal ion suppression and enhancement was observed, and no matrix or drug interferences were detected, including paynantheine, speciogynine, speciociliatine, and mitraphylline (Cayman Chemical, Ann Arbor, MI).

Liquid Chromatography MS/MS Quantitation

The mobile phases were organic (B) 100% acetonitrile with 0.1% formic acid and aqueous solvent (A) 100% ultra-pure water with 0.1% formic acid. Liquid chromatography method started at initial flow at 0.4 mL/min, 95% A with linear ramp to 55.1% A to 7.25 minutes. Additional linear ramp at 1 mL/min to 5% A to 7.75 minutes and held until 9.75 minutes. Flow was changed to initial mobile phase and held for 2 minutes to re-equilibrate prior to next injection. Method used Phenomenex Kinetex® Column, 1.7 µm Biphenyl Å, 2.1 × 100 mm kept at 40°C with 7.5 µL in PLNO mode for injection volume.

Extraction procedure of the method included a volume of 0.5 mL for sample, standards, and blank, 0.5 g was used for liver and gastric contents. An addition of 0.1 mL of internal standard (1000 ng/mL mitragynine-d_3) was added before vortex mixing and centrifuging at 2500 rpm. Supernatant was transferred into clean tube with 0.2 mL of ultra-pure water with 5% formic acid. The solution was vortex mixed and aspirated two times with DPX®WAX-S tips using pneumatic extractor. A volume of 0.1 mL of the organic layer was diluted with 0.9 mL of ultra-pure water in the LC vials. Calibration curve for mitragynine was from 10 to 640 ng/mL with quality control standards at 30 and 300 ng/mL weighted with a quadratic $1/x^2$, with no forcing through the origin. Quantitation method was previously validated for blood, urine, and tissue following ANSI-ASB guidelines (6, 7). Method validation showed no matrix interferences or drug interferences, no significant ion suppression or enhancement, and bias and precision showed to be within 20% of every matrix except for brain tissue. Full validation information was reported in Mata and Davis (2022).

CASE HISTORY

In January of 2021, a 32-year-old Caucasian male was brought into a Southern California hospital by

Table 1: Toxicological Results.

Drug	Cardiac blood (mg/L)	Femoral venous blood (mg/L)	Liver (mg/kg)	Gastric contents (mg)
Mitragynine	7.5	3.3	42.2	33.1
7-Hydroxymitragynine	Detected	Not analyzed	Not analyzed	Not analyzed

ambulance after he was witnessed having seizures and collapsing after dinner at his drug rehabilitation center at approximately 07:40 hours. He was asystolic in the field and when he arrived at the emergency department at 07:57 hours after being transported by emergency medical services. He was given two doses of Narcan in the field without effect. In the emergency room, he was given sodium bicarbonate, calcium chloride, and epinephrine but spontaneous circulation never returned, and he was pronounced dead at 08:11 hours. No other signs of trauma were observed beyond a superficial laceration above his right eyebrow. Upon investigation, it was found that he had a suspended driver's license due to driving under the influence of alcohol and multiple arrest records with various law enforcement agencies.

No one at the recovery residence admitted to witnessing the decedent ingest any drugs and no drug paraphernalia samples were submitted to the death investigator. When speaking to relatives, it was explained that he had previously abused alcohol, illicit drugs, and prescription opioid medication, but had been approximately one-year sober. He also had a psychological history of severe depression and oppositional disorder. His previous medical history included back pain, which was attributed to his weight, and headaches. Three to four months prior to his death, he reported to his mother that he had suffered from a seizure, but no medical records could be found to corroborate this. He had attempted suicide multiple times in the past including trying to hang himself eight times, intentionally overdosing, and cutting his wrists. The most recent suicide attempt known to family members was February of 2020.

AUTOPSY FINDINGS

The decedent weighed 296 pounds and was 6 feet 4 inches tall. Besides a small laceration above his eye, there were no noticeable injuries on the body. Autopsy findings noted cardiomegaly with the heart weighing 550 g and left ventricular hypertrophy at 2.0 cm and the autopsy was performed 50 hours after death. Postmortem examination revealed severe hepatomegaly with a liver weight of 4150 g. The decedent's lungs were congested with each weighing 650 g and there was cerebral edema with a brain weight of 1620 g. Cardiac blood, femoral venous blood, liver, brain, vitreous humor, urine, and gastric contents samples were sent to the toxicology laboratory for analysis.

TOXICOLOGICAL ANALYSES

The toxicological results can be found in **Table 1**. 7-Hydroxymitragynine was detected qualitatively in the central blood. No other drugs, including prescription or illicit were detected including ethanol.

DISCUSSION

Following a thorough medicolegal autopsy, comprehensive postmortem toxicology, and in-depth scene investigation, the cause of death (COD) was due to acute mitragynine intoxication combined with cardiomegaly with left ventricular hypertrophy, with severe hepatomegaly and obesity listed as other significant contributing conditions. The mode of death was determined to be an accidental overdose.

In comparison to other reported mitragynine death cases, the age and gender, majority being male, were similar to what was previously found (5, 8-17). For age of decedents in mitragynine-related deaths, Neerman et al. (2013) reported the youngest decedent who was a 17-year-old male and the two oldest decedents were both female at 60 and 65 years old from Mata and Andera (2020) and Wang and Walker (2018), respectively.

For reported cases with tissue weight, combined lung weight was similar to that found in this case as <1000 g, combined weight (5, 8, 10, 12). However, many of the cases reported also included other drugs such as opioids or benzodiazepines, which could also contribute to an increased lung weight. In both mitragynine-only death investigation cases that Behonick et al. (2022) reported, each had lung weight greater than 1000 g and pulmonary edema.

The largest range of mitragynine concentrations, 0.0056 to 29 mg/L, was reported by Papsun et al. (2019) but included not just medicolegal death investigation cases but also driving under the influence of drug cases, clinical cases, and drug-facilitated sexual assaults. The cardiac blood concentration in this case is higher than those reported by Mata and Andera (2020); however, the peripheral blood falls within the range from their reported cases. Behonick et al. also reported on two cases with no other significant toxicological findings, and both had concentrations around that of the peripheral blood in this case, 2.325 and 3.809 mg/L. Domingo et al. (2017) discussed 2 cases in which mitragynine was present at 0.79 mg/L and 0.010 mg/L in the femoral blood. These concentrations are significantly lower than was detected in this case and both cases had other drugs detected and CODs that did not include mitragynine. Besides this case and the two reported by Behonick et al., all cases had other significant toxicological findings that did not include mitragynine.

Matson and Schenk (2019) also had a case with limited other toxicological findings, the peripheral blood concentration of 1.9 mg/L of mitragynine with 0.0026 mg/L of THC and qualitatively positive for caffeine, cotinine, and naloxone. Neerman's decedent had 0.60 mg/L of mitragynine in the femoral blood. The data in the study by McIntyre et al. (2018) had central blood (0.19 mg/L) and peripheral blood (0.23 mg/L). Wang published a case report of a 65-year-old female who had a history of chronic obstructive pulmonary disease and was taking lorazepam and oxycodone. The femoral venous blood contained concentrations of mitragynine (2.5 mg/L), with lorazepam (0.063 mg/L) and oxycodone (0.19 mg/L). Postmortem redistribution (PMR) occurs when the concentration of a drug changes between time of death and autopsy because of passive diffusion from drug deposits in organs. This diffusion can occur for several reasons, such as pH changes after death, drug lipophilicity, and volume of distribution (18). The PMR for this case is 2.3, with a postmortem interval of 50 hours. This is higher than any case reported by Mata and Andera who had a range of 0.04 to 1.26 with a mean and median of 0.75 and 0.79, respectively. No other information on mitragynine PMR could be found in the literature and more work needs to be done to determine if mitragynine does have PMR.

The liver concentrations in this case were higher than those previously reported (5, 13, 14). The amount of gastric contents fell within the range previously reported by Mata and Andera. No other liver or gastric content concentrations could be found in literature at the time of writing; however, Holler et al. (2011) reported concentrations found in several other common toxicology matrices.

Seizures are often observed after mitragynine has been ingested and this decedent was witnessed having a seizure prior to being pronounced dead. Afzal et al. (2022) reported a clinical case of seizures from a person who had a history of drinking 3 to 4 bottles of 7 mL kratom every week for six months. The patient was taken to the hospital after having a seizure and he survived the ingestion. No samples were available for analysis according to the report.

The majority of mitragynine-related deaths typically have other drugs present that are also significant or contributory to the accidental drug poisoning. Behonick et al. (2022) reports the only other cases where mitragynine is the sole toxicological finding and the COD was acute mitragynine intoxication, similar to this reported case.

CONCLUSION

We report a single case where mitragynine is listed as the sole agent to have caused an accidental overdose. Mitragynine concentrations are given in central blood (7.5 mg/

L), peripheral blood (3.3 mg/L), liver (42.2 mg/kg), and gastric contents (33.1 mg) to contribute to the little known information about drug distribution. Concentrations in blood are similar to those given in other death investigation cases involving mitragynine. 7-Hydroxymitragynine was analyzed for and qualitatively identified only in the central blood. For future work, obtaining concentrations for 7-hydroxymitragynine, along with other constituents of the *Mitragyna speciosa* plant, and comparing them to mitragynine in fatal cases would be beneficial for public health where mitragynine is concerned.

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