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Case Report

An accidental poisoning with mitragynine

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ABSTRACT

An increasing number of drugs of abuse are sold word wide over the internet. Names like "legal highs", "herbal highs" etc. give the impression that these are safe products, although the risk of fatal reactions might be substantial. Leaves from the plant *Mitragyna speciosa*, contain active compounds like mitragynine and 7-hydroxymitragynine. It has been reported that the potency of 7-hydroxymitragynine at the μ-opioid receptor is 30 times higher than that of mitragynine and 17 times higher than that of morphine. Case reports regarding poisoning with Kratom are reported, but the toxic or lethal ranges for the concentrations of the active substances have not been established, and concentrations of 7-hydroxymitragynine have not been reported previously.

We present a case report where a middle aged man was found dead at home. The deceased had a history of drug abuse and mental illness for several years. At autopsy, there were no significant pathological findings. Post-mortem analysis of peripheral blood revealed: zopiclone 0.043 mg/L, citalopram 0.36 mg/L and lamotrigine 5.4 mg/L, i.e. concentrations regularly seen after therapeutic ingestion of these drugs. Additionally mitragynine 1.06 mg/L and 7-hydroxymitragynine 0.15 mg/L were detected in blood and both also in urine.

The high concentrations of mitragynine and 7-hydroxymitragynine indicate that the cause of death is intoxication by these substances; and the circumstances point toward the manner of death being accidental. We recommend that both mitragynine and 7-hydroxymitragynine are analyzed for in cases with suspected Kratom intoxication.

1. Introduction

The number of available drugs encountered over the Internet are increasing, and names like "legal highs" and "herbal highs" could make the consumer believe these are safe and natural products [1]. The toxicity of these products is not known, and the amount of active substances ingested can vary widely, and constitute a major risk of fatal toxicity.

Mitragyna speciosa Korth (Rubiaceae) is a tropical tree that is commonly found in Southeast Asia. Leaves from this plant, known as "Kratom" in Thailand and as "Biak-Biak" in Malaysia, have stimulant effects in low doses and sedative and opioid-like effects after ingestion of high doses [2,3]. Thai and Malaysian natives have traditionally consumed the leaves by chewing, smoking or drinking them as tea [4]. Mitragynine is considered to be the major constituent in the plant, and is responsible for the opioid effects through the μ-receptor [5]. "Kratom" has been widely used as an opium substitute during opium withdrawal, as well as for pain relief [4]. "Krypton" is an herbal mixture containing powdered "Kratom" leaves and O-desmethyltramadol as a synthetic additive, and several deaths have been reported after ingestion of this drug [6,7].

Kikura-Hanajiri et al. [8] measured the contents of mitragynine and the minor alkaloid 7-hydroxymitragynine in "Kratom" products distributed as "incense" on the drug market, and mitragynine concentrations ranged from 1% to 6% and 7-hydroksymitragynine from 0.01% to 0.04%. From in vitro experiments and animal models, the potency of 7-hydroxymitragynine is reported to be 30–46 times higher than mitragynine [3,9–11] and 17 times higher than morphine [9]. The toxic mitragynine concentrations in humans are poorly defined, and no toxic or lethal ranges have been established. The structural formulae of mitragynine and 7-hydroxymitragynine are presented in Fig. 1.

Studies have reported that *Mitragyna speciosa* preparations have analgesic, antipyretic, antidiarrheal, euphoric, anti-depressant, and anxiolytic effects, and the preparations have been used

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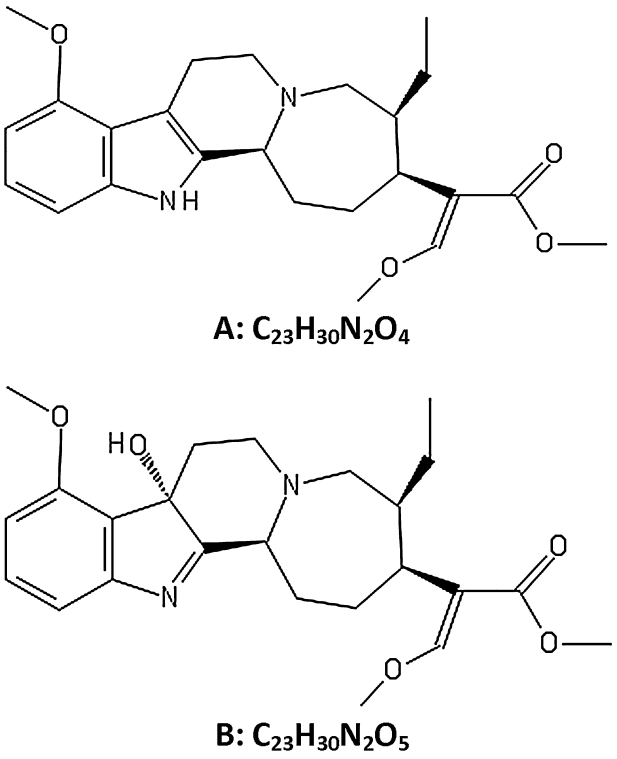

Fig. 1. shows the structural formulae of mitragynine (A) and 7-hydroxymitragynine (B).

as opium substitution. It is also reported that they can cause anorexia, dryness of the mouth, decreased diuresis and constipation after long term use in of high doses [12]. Mitragynine is shown to induce condition place preference in rats, which indicates an abuse potential [13]. Abuse of "Kratom" by drug addicts constitutes a major concern in countries like Malaysia and Thailand, and has been listed as a control item, in contrast to other parts of the world, where this is not regulated [4].

A few case reports of fatal intoxications involving mitragynine have been published. Holler et al. [14] described a fatality involving mitragynine in combination with propylhexedrine, a potent α-adrenergic sympathomimetic amine found in nasal decongestant inhalers. Kronstrand et al. [7] reported nine cases of fatal intoxications with "Krypton" in Sweden, during a period of less than one year. A drug fatality case involving "Kratom" has also been reported by Neerman et al. [15], where dextromethorphan (an antitussive), diphenhydramine (an antihistamine), and the benzodiazepines temazepam and 7-amino-clonazepam (metabolite of clonazepam) were detected simultaneously.

We present a case of fatal intoxication with mitragynine in combination with zopiclone, citalopram and lamotrigine. Concentrations of mitragynine and 7-hydroxymitragynine both in blood and urine are reported.

2. Case report

A middle aged man with a history of substance abuse as well as psychiatric disease was found dead in his bed. Because of his drug habit, he had been subjected to drug testing at work. In order to avoid testing positive, he had bought "Kratom" on the internet. The substance was mixed with water and ingested orally. He had commented that the most recent batch was different from, and possibly more potent than, what he had received previously. The afternoon before he died, his family perceived him as unwell and clearly intoxicated and after going to bed they had heard him snoring. The following morning, he was found dead in his bed.

3. Autopsy findings

A medicolegal autopsy was performed 3 days post mortem. The deceased was overweight (BMI 35). No injection marks were found. There were patchy areas of bronchopneumonia. Furthermore, the lungs were congested and oedematous. His heart was somewhat enlarged and a fibrotic scar was observed in the anterior wall. There was a moderate degree of coronary atherosclerosis and a stent in the left anterior descending artery. There were some superficial ulcerations in the gastric mucosa but no signs of significant blood loss.

The results of the toxicological analyses are described below. The cause of death was considered to be intoxication with "Kratom", possibly in combination with the other substances detected. Pneumonia was considered to be precipitated by the intoxication and to have contributed to the fatal outcome.

4. Materials and methods

4.1. Analytical toxicology

Whole blood from the femoral vein and urine were collected at autopsy in 25 mL Steriline® tubes (Bibby Sterilin, Staffordshire, UK). The sample tube contained 0.3 mL 67% (w/v) potassium fluoride solution as preservative.

The post-mortem blood sample was screened for a selection of benzodiazepines, z-hypnotics, opioids, psychostimulants and THC by ultra-performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) [16], and also for medicinal drugs including antidepressants, antipsychotics, analgesics and antiepileptics using the same technique. Screening analysis for blood ethanol was performed using a head-space gas chromatography equipped with flame ionization detector (HSGC-FID) [17]. Information from the case indicated that the deceased had taken mitragynine or other synthetic psychoactive substances. The blood sample was also analyzed by UPLC–MS/MS for a selection of psychoactive compounds, including mitragynine.

The urine sample was screened by an immunological method using an AU680 instrument from Beckman Coulter (Beckman Coulter Inc., CA, USA) for a standard selection of drugs of abuse (amphetamines, barbiturates, buprenorphine, benzodiazepines, cannabis, phencyclidine, cocaine, methadone and opiates). The urine was also screened for ethanol by the same instrument using an enzymatic method (alcohol dehydrogenase) [18].

4.2. Determination of mitragynine and 7-hydroxymitragynine

Mitragynine, 7-hydroxymitragynine and amphetamine-d_{11} (internal standard) were supplied by Cerilliant® (Austin, TX, USA). Methanol (MeOH, HPLC-grade) and acetonitrile (ACN, far UV HPLC) were purchased from LAB-SCAN (Dublin, Ireland). GPR Grade formic acid (98%, HCOOH) and sodium chloride (NaCl) were supplied by VWR (VWR International AS, Oslo, Norway). Deionized water was obtained from a Milli-Q UF Plus water purification system (Millipore, Bedford, MA, USA). Human whole blood was supplied by the Blood Bank at Oslo University Hospital, Ullevaal, Norway and urine by the staff at the Norwegian Institute of Public Health, Division of Forensic Sciences, Oslo, Norway.

Stock solutions of mitragynine and 7-hydroxymitragynine were prepared in methanol. Working standards were prepared in water containing 0.9% NaCl. Five calibration samples were prepared from whole blood spiked with working standard solutions (0.050–1.6 mg/L for mitragynine and 0.052–1.7 mg/L for 7-hydroxymitragynine). Quality control (QC) samples were prepared independently at two concentration levels (0.080 and 0.80 mg/L for mitragynine and 0.083–0.83 mg/L for 7-hydroxymitragynine).

To an aliquot of 100 μL whole blood, 50 μL of internal standard solution containing amphetamine-d_{11} (0.7 mg/L) and 300 μL ACN/MeOH (85/15, v/v) mixture was added. The samples were immediately agitated for 1 min and thereafter put in a deep freezer for a minimum of 10 min. The samples were centrifuged at 4500 rpm (3900 × *g*) for 10 min at 4 °C. 50 μL from ACN/MeOH layer was transferred to the autosampler vials and diluted with 100 μL water. The urine sample was analyzed against working standards after dilution with water, without hydrolysis.

The samples were analyzed in accordance with a previously published UPLC-MS/MS method [16] on a Waters ACQUITY UPLC-system (Waters Corporation, Milford, MA, USA), applying an Acquity HSS T3-column 100 mm × 2.1 mm I.D. (Waters Corporation, Milford, MA, USA), with an average pore size of 100 Å and a particle diameter of 1.8 μm. The mobile phases consisted of A: 10 mM ammonia formate buffer, pH 3.1, and B: methanol. A Waters Quattro Premier XE tandem mass spectrometer, equipped with a Z-spray electrospray interface, was used for all analyses. Positive ionization was performed in the multiple reaction monitoring (MRM) mode, with two transitions for mitragynine (399.1 > 174.0 and 399.1 > 238.0) and 7-hydroxymitragynine (415.1 > 190.0 and 415.1 > 238.0) and one transition for amphetamine-d_{11} (147.0 > 98.0). Quantification was performed with TargetLynx using MassLynx 4.1 soft-ware. The retention times were 3.34, 3.31 and 2.12 min for mitragynine, 7-hydroxymitragynine and amphetamine-d11, respectively. The calibration curves were linear with correlation coefficients greater than 0.995 for both analytes. The QC-samples (two replicates at each level) had less than 11% deviation from nominal values for both analytes. The lowest calibrator had S/N-ratios > 10 for all quantifier ions and S/N-ratios > 4 for all qualifier ions.

5. Toxicological findings

Routine toxicological analyses revealed zopiclone (0.043 mg/L) [Lethal level: 0.6 mg/L], citalopram (0.36 mg/L) [L: 5.0 mg/L] and lamotrigine (5.4 mg/L) [L: 50 mg/L] [19] in post-mortem whole blood. No other compounds, including O-desmethyltramadol, of the standard analytical program were detected.

Mitragynine (1.06 mg/L) and 7-hydroxymitragynine (0.15 mg/L) were found in blood after a more comprehensive analysis. In urine the concentrations of mitragynine and 7-hydroxymitragynine were 3.47 and 2.20 mg/L, respectively.

6. Discussion and conclusion

In this case, a high concentration of mitragynine was detected in whole blood, as well as 7-hydroxymitragynine at a lower concentration level. The concentrations of zopiclone, citalopram and lamotrigine (all CNS depressants) were within therapeutic concentration ranges. Mitragynine intoxication was assumed to be the main cause of death. As 7-hydroxymitragynine is several times more potent than mitragynine, this substance is likely to have played a major part in causing death. It is reported that the combination of "Kratom" and central nervous system depressants might cause respiratory depression [5] and ingestion of such substances may thus have contributed to death.

The toxicity of mitragynine in humans is poorly defined, and no toxic or lethal ranges have been established. Kronstrand et al. [7] found mitragynine levels in nine cases that varied between 0.02 and 0.18 μg/g. Holler et al. [14] and Neerman et al. [15] found mitragynine concentrations of 0.39 mg/L and 0.60 mg/L, in post-mortem blood samples. The concentration in our case of 1.06 mg mg/L in peripheral blood is thus higher than previously reported concentrations.

The concentrations of mitragynine and 7-hydroxymitragynine in urine in our case were 3.47 and 2.20 mg/L, respectively. Holler et al. [14] reported a mitragynine concentration of 1.20 mg/L in a hydrolyzed urine sample. A mitragynine concentration of 0.17 mg/L in urine from a hospitalized patient, who used "Kratom" regularly to self-medicate chronic pain, has been reported by Nelsen et al. [20]. Whether or not the samples were hydrolyzed, is not stated. The concentration of free mitragynine in our case is thus higher than previously reported. To our knowledge, concentrations of 7-hydroxymitragynine in human samples have not been reported previously, but considering the potency of 7-hydroxymitragynine, this should be analyzed.

The other toxicological findings in the reported by Neerman et al. [15] were dextromethorphan (an antitussive drug), diphenhydramine (an antihistamine), and the benzodiazepines temazepam and 7-aminoclonazepam (metabolite of clonazepam). The forensic pathologist certified the cause of death as possible "Kratom" intoxication. In our case, concomitant findings of supposedly therapeutic levels of zopiclone, citalopram and lamotrigine were seen. The post mortem concentration levels of these drugs, together with the information that the deceased used these substances on a regular basis point toward these medicinal drugs being of little significance in causing death in this case. It can however not be excluded that these drugs may have enhanced the effects of mitragynine and 7-hydroxymitragynine.

More than 40 different alkaloids have been isolated from the "Kratom" leaves, but studies have focused on the effects of mitragynine, being the predominant alkaloid [2]. Dose-dependent effects have been reported, and might be due to the different psychoactive compounds [5]. Mitragynine has higher affinity to the μ-opioid receptor than morphine and has been considered to be responsible for the substance's opioid effects [5], but the μ-opioid receptor's affinity for the minor constituent 7-hydroxymitragynine is even higher [3]. Analysis of the latter is thus important, in cases where intoxication with *Mitragyna speciosa* is suspected. There are some reports on the metabolism of mitragynine [5], but little is known about the effects of the metabolites of *Mitragyna speciosa* [2]. It has been suggested that mitragynine might be metabolized into 7-hydroxymitragynine [21]. More information regarding the degree of this metabolism would however provide important information regarding the toxicity of this plant. Manda et al. [22] have reported that mitragynine is metabolically stable in human liver microsomes, while 7-hydroxymitragynine is converted to mitragynine (45%) with a half-life of 24 min.

Both Kronstrand et al. [7] and Holler et al. [14] reported findings of pulmonary edema ("heavy lungs") or pulmonary congestion. This was also found in the medicolegal autopsy in our case, and is common in opioid overdoses. Seizures have also been reported after consumption of mitragynine [12], but there is no information of this in our case. Mitragynine and 7-hydroxymitragynine both have high affinity to the μ-opioid receptor, and the respiratory depression and subsequent pulmonary congestion seen in our case, might be due to μ-opioid receptor activation.

The high concentrations of mitragynine and 7-hydroxymitragynine found in blood are likely to have caused death in our case, which was considered to be an accidental poisoning. Both substances should be analyzed in such cases.

Ethics

Approval to publish this case report has been given by the Public Prosecutor and the Council of Confidentiality and Research (appointed by the Norwegian Ministry of Justice).

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