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Case Report

Mitragynine concentrations in two fatalities

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ABSTRACT

Two cases of fatalities are reported of which the recreational use of *Mitragyna speciosa* ("kratom") could be confirmed. One of these cases presents with one of the highest postmortem mitragynine concentrations published to date. Our results show that even extremely high mitragynine blood concentrations following the consumption of kratom do not necessarily have to be the direct cause of death in such fatalities as a result of an acute overdose. The two cases are compared with regard to the differences in mitragynine concentrations detected and the role of mitragynine in the death of the subjects. Irrespective of the big differences in mitragynine concentrations in the postmortem blood samples, mitragynine was not the primary cause of death in either of the two cases reported here. Additionally, by rough estimation, a significant difference in ratio of mitragynine to its diastereomers in the blood and urine samples between the two cases could be seen.

1. Introduction

An ongoing emergence of psychoactive substances is taking place and over the last few years even the worldwide distribution of so-called "legal highs" over the internet has become more common. An example of such substances that remain uncontrolled in most countries and of which the use continues to increase, is the naturally occurring indole alkaloid, mitragynine [1–4].

Mitragynine is one of the main psychoactive components of *Mitragyna speciosa* ("kratom"), a tree indigenous to New Guinea, the Philippines and Southeast Asia, particularly Malaysia, Thailand and Indonesia. The kratom plant is known to contain more than twenty five different alkaloids, among which are the mitragynine diastereomers speciociliatine, speciogynine and mitraciliatine. These three substances differ only in the orientation of the chiralic carbons 3, 20 or both with respect to the main alkaloid (see Fig. 1) [5–8]. As a result of these minor differences the potency of speciociliatine is thought to be approximately thirteen fold lower than that of mitragynine, whereas not much is known about the pharmacological activity of speciogynine and the less abundant mitraciliatine [9]. The 7-hydroxy derivative of mitragynine, on the other hand, has a more than 40-fold higher potency than that of mitragynine [10,11]. Yet the narcotic, stimulant and other dose-dependent effects of kratom have been attributed primarily to mitragynine [12,13].

The substance shows a unique concentration dependent pattern in that it demonstrates cocaine-like stimulating effects at low doses and opioid-like activity, such as sedation, at higher concentrations [14,15]. Its pharmacological activity is very similar to that of morphine, albeit approximately thirteen times more potent than morphine. In addition to its almost full agonistic activity on the μ-opioid receptors, mitragynine also acts as agonist on both the δ- [16] and κ-opioid-receptors [12,17,18]. Moreover, postulations of its noradrenergic and serotonergic activity have also been reported [12]. Nevertheless, the pharmacological effect of kratom may fundamentally be determined by the total alkaloid composition [19], which is highly dependent on geographical, chronological and morphological factors.

For one, the area and climate of cultivation play an important role [9,20–23], so much so that even the geographical location within the same country may cause variations in alkaloid constitution [21,24]. For example, in a study to analyze exactly this, Orio *et al.* [23] showed that plants with Indonesian origin contain the highest amount of mitragynine and related alkaloids when compared to plants from Malaysia and Thailand. The latter contain a higher amount of alkaloids than the Malaysian plants, albeit a very similar alkaloid distribution. This is also in accordance

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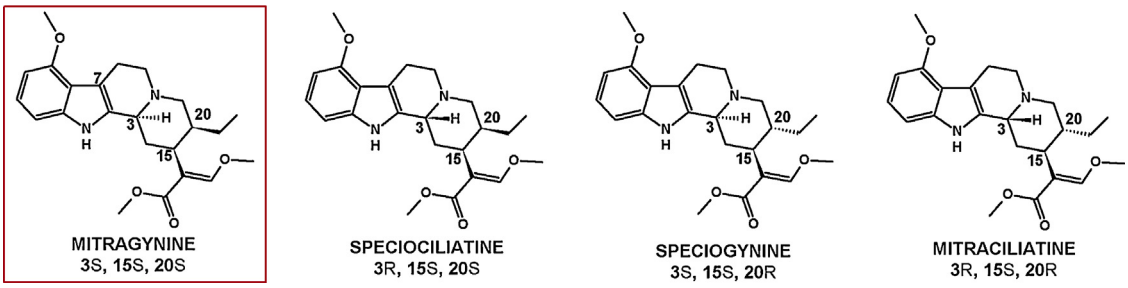

Fig. 1. Chemical structures of mitragynine and some of its diastereomers, showing the differences in stereochemistry.

with earlier reports, which state that the Malaysian *Mitragyna speciosa* leaves contain only between 12 and 25% mitragynine of the crude base [25,53], whereas leaves from Thailand yielded approximately 66%. The diastereomers speciogynine and speciociliatine in the Thai plant yielded merely 6.6% and 0.8% respectively [19]. 7-Hydroxymitragynine makes up approximately 2% of the total plant content, irrespective of its geographical origin. Studies have shown that plants cultivated in non-indigenous areas have substances other than mitragynine as main alkaloid [22]. This is also the case for the younger plants of which, in contrast to mature plants, mitragynine only makes up a minor component [11,22,26–28].

Depending on the alkaloid constitution, one can distinguish between white vein, red vein and green vein kratom. Kratom red vein (see Fig. 2a), for example, is solely a product of Thailand. As is to be expected from the differences in alkaloid pattern, the three types of strains differ with regard to their pharmacological effects. According to internet drug forums, red vein kratom is known to be more sedative, whereas green and white kratom are the more stimulating strains [29–33].

The differences in alkaloid composition may be reflected in the illegal products from different distributors, which in turn is likely to contribute to differences in the overall pharmacological properties of the mixtures. In a study of the constitution of illegal kratom products, Schröfel *et al.* [34] analyzed a large number of samples that originated mainly from private purchases for the purpose of product monitoring. The exact origin of the individual samples are however unknown. Their results confirm that much variation exists between the ratios of selected alkaloids among different herbal mixtures.

The herbal mixtures, which are usually purchased over the internet, are generally abused mainly for the euphoric effects. The use of mitragynine, however, dates back several decades. The natives of indigenous areas traditionally use the substance as a stimulant, as a narcotic, or as substitute for opiate addiction [35–38]. Typical routes associated with the consumption of kratom include chewing and swallowing of the leaf in its fresh form, or by drinking the tea prepared from dried leaf powder [39]. Occasionally, the dried leaves or resin are smoked and lately even kratom capsules can be purchased over the internet [15].

Similar to other illegal drug preparations, a toxic potential should be associated with mitragynine use. Yet, only few reports of quantified *M. speciosa* intoxications have been published to date [4,40–44] and specifications with regard to toxic and lethal mitragynine doses in humans are yet to be established. The reported concentrations of mitragynine in postmortem blood samples range from 230 μg/L [44] to 1006 μg/L [4]. However, in all of these cases at least one other substance with an additional depressing effect on the central nervous system could be detected.

Thus, although some cases have been reported of deaths attributed to kratom use, no solid evidence has yet been provided where the substance was the sole contributor to the fatality [32]. As a result, the unavailability of reference data on blood concentrations makes it particularly difficult for forensic toxicologists to interpret the exact role of mitragynine concentrations in *M. speciosa* related intoxications. For this reason we now report two cases of mitragynine related deaths, one of which describes one of the highest postmortem blood concentrations published to date. With this we wish to show that even an extremely high mitragynine concentration, such as the one we report here, does not necessarily have to be the primary cause of death. Hence, although both cases reported here are related to the recreational use of mitragynine, the substance itself was likely not the direct cause of death as a result of an acute overdose.

The main goal of this report is thus to contribute to literature regarding mitragynine concentrations in fatal cases, irrespective whether mitragynine was the direct cause of death or merely a contributing factor. We also address the differences in ratio of

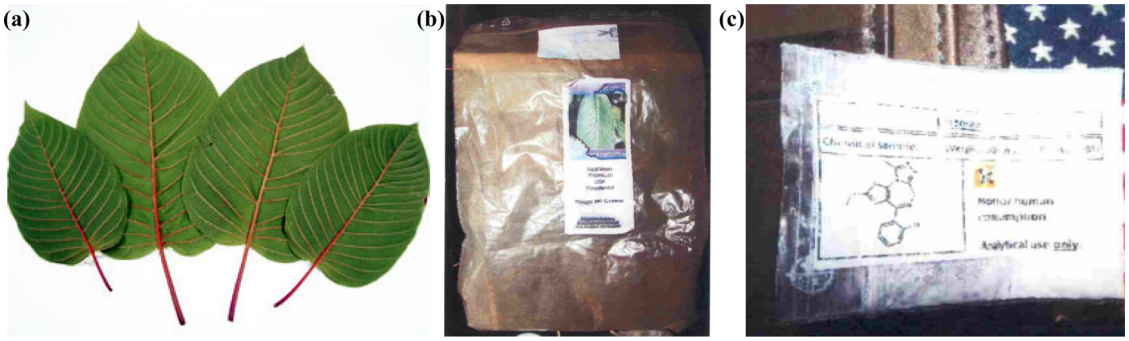

Fig. 2. a) Leaves of the kratom plant, from which red vein kratom can be isolated [29] and pictures of exhibits found at the scene of case 1: (b) packaged red vein kratom (labeled with: *Kratom*; *Red Vein*; *Premium*; *USA*; *Powdered*; "*Menge*" 100 gm) and (c) sachet with etizolam (labeled with chemical structure and the following indication: *Not for human consumption*; *Analytical use only*).

mitragynine to its diastereomers in the analyzed blood and urine samples between the two different cases.

2. Case histories

2.1. Case 1

A 22-year old male was found dead in his bed on the morning following the consumption of an herbal mixture. The subject followed a treatment program for psychosis and anxiety as a result of a well-known drug addiction. According to the father of the deceased, his son mixed an unknown amount of the herbal substance (which he supposedly ordered from the internet) with water and then drank it together with an unknown tablet. This was reportedly followed by an incident, during which the deceased fell from a window from the first floor before going to bed. The subject refused medical treatment, despite presumably intense pain as a result of the fall.

Upon discovery of the corpse the following morning, a red-brown colored secretion could be noted on the subject's cheek. About half of an original 100 g package of "Red Vein", a plastic sachet with etizolam (see Fig. 2) and fluoxetine were found at the scene.

Standard procedures were applied for the medico-legal postmortem investigation. The corpse showed signs of decomposition. In correspondence with the case history, a haematoma and humerus fracture of the left arm could be confirmed during the postmortem examination. Intracranial pressure and a mild case of pulmonary edema could be detected. The cause of death was determined to be the aspiration of chyme by the subject, possibly due to a loss of consciousness. A completely filled bladder with approximately 500 mL urine was found. A preliminary test on the urine of the subject tested positive for benzodiazepines.

2.2. Case 2

The second case is of a 20-year old male who was known to suffer from autism. A disposable syringe was found in the right hand of the corpse and various puncture wounds were visible on the left arm. Drug paraphernalia, such as utensils for illegal drug abuse, butane-1,4-diol and a bag with approximately 250 g of a brown-colored powder could be found on the scene. The latter substance, which contained the inscription *Kratom*, was likely purchased over the internet. The particular strain of kratom, the route of administration, as well as the time of consumption remain unknown.

Standard procedures were applied for the medico-legal postmortem investigation. During the autopsy, urinary retention could be identified (the bladder contained approximately 650 mL urine), with the corpse itself showing signs of decomposition. Signs of intracranial pressure could be identified. No clearly defined cause of death could be derived from the postmortem examination alone and a suspicion of drug intoxication was suggested by the medical examiner based on the case history.

3. Materials & methods

3.1. General toxicological analysis

Standard procedures were followed for collection of urine from the bladder shortly before dissection and extraction of whole blood from the femoral vein during the autopsy of both subjects. Both autopsies took place two days after discovery of the corpse. Blood and urine samples, among other biological materials for routine toxicological screenings, were stored at −20 °C prior to analysis. Blood and urine samples for ethanol determination were stored at 2–8 °C. Validated liquid and gas chromatography coupled mass spectrometry procedures, performed at the Forensic Toxicological Centre (FTC) in Munich and at the Department of Toxicology, Institute for Forensic Medicine of the University of Munich, were applied for these analyses. Only substances detected in femoral blood samples were quantified during general screening procedures. Based on circumstantial evidence and case history (1,4-butanediol found on the scene), additional analyses for the detection of γ-hydroxybutyric acid (GHB) were performed on the biological material of case 2.

3.2. Determination of mitragynine concentrations and stereoisomer screening

Femoral blood and urine samples were used for both the quantification of mitragynine, as well as for the stereoisomer screening at FTC-Forensic-Toxicological Laboratory GmbH in Vienna, Austria. For this purpose a standard procedure, validated for quantifying a large number of drugs in human plasma/serum and urine by liquid chromatography–tandem mass spectrometry (LC–MS/MS) was applied. A sample aliquot (100 μL of blood or 20 μL of enzymatically hydrolyzed urine in 80 μL hydrolysis buffer) was mixed with 10 μL of multi-internal standard solution and 1 mL acetonitrile. After mixing and centrifugation 100 μL of the supernatant were removed, to which 5 μL of 50% (v/v) aqueous formic acid were added. The solution was evaporated at ambient temperature under a gentle stream of nitrogen. The residue was reconstituted in 200 μL of a solution of 5 mM ammonium formate and 0.1% formic acid in water/methanol (85:15; v/v). Liquid chromatography was performed on a Kinetex™ Biphenyl column (Phenomenex®, Aschaffenburg, Germany). A binary linear gradient with 5 mM ammonium formate and 0.1% formic acid in water, as well as 5 mM ammonium formate and 0.1% formic acid in methanol were used as mobile phases. Electrospray ionization in positive ion mode and retention-time dependent selected reaction monitoring mode (SRM), with two diagnostic fragmentation pathways (399.2/174.0; 399.2/159.0), was used during MS/MS analysis for the identification and quantification of mitragynine. From the set of routine internal standards, 5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH) was used as internal standard owing to its closest similarity of retention time to that of mitragynine in this method. The calibration range spanned 2–80 μg/L for blood and 10–400 μg/L for urine. Blood samples with mitragynine concentrations exceeding the highest calibration level were reprocessed after appropriate dilution.

The discovery of additional chromatographical peaks with a similar mass as that of mitragynine pointed towards the presence of diastereomers. For confirmation, screening of mitragynine diastereomers was performed, during which three additional MS/MS transitions were monitored in non-retention time dependent SRM mode.

3.3. Ethanol determination

The headspace gas chromatography and the enzymatic alcohol dehydrogenase method for the determination of alcohol concentrations were conducted on blood and urine samples. All measurements were done in duplicate and an average was calculated from the four obtained values for each sample.

4. Results

4.1. General toxicological analysis

4.1.1. Case 1

Substances detected in femoral blood and urine relevant to the death of case 1 are indicated in Table 1, among which was a methylmethcathinone derivative. No reference substance was

Table 1
Relevant substances detected in case 1 in femoral blood and urine, respectively. The concentrations detected in femoral whole blood, as well as reference toxic blood concentrations are indicated [45–47].

Case 1			
Femoral blood			Urine
Substance	Concentration detected	Lowest toxic concentration	Substance
Mitragynine	790 μg/L	Unknown	Mitragynine (>400 μg/L)
Mitragynine diastereomers	Not quantified	–	Mitragynine diastereomers
Etizolam	280 μg/L	30 μg/L	Etizolam
Pregabaline	3 mg/L	10 mg/L	Pregabaline
Pipamperon	7.4 μg/L	600 μg/L	Pipamperone
Lorazepam	6.9 μg/L	300 μg/L	Lorazepam & a degradation product
Triazolam	1.1 μg/L	40 μg/L	A triazolam metabolite
Fluoxetine	89 μg/L	1500 μg/L	Fluoxetine
Quetiapine	18 μg/L	1000 μg/L	Quetiapine & metabolite
Olanzapine	5.8 μg/L	200 μg/L	Olanzapine
(Likely) 2-MMC	~5.2 μg/L	–	–

available for the latter measurement. However, the retention time of the substance points towards the 2-methylmethcathinone (2-MMC) derivative.

Unspecified mitragynine diastereomers were detected in blood and urine, of which the identification was based on their mass transition patterns. The respective concentrations of the diastereomers were, however, not quantified (see Section 4.2).

4.1.2. Case 2

The substances detected in femoral blood and urine which are relevant to the death of case 2 are indicated in Table 2.

4.2. Mitragynine diastereomers

Fig. 3 below shows the chromatograms from blood and urine samples of cases 1 and 2, respectively, indicating differences in the ratio of mitragynine to its diastereomers. The exact amounts of diastereomers in each sample were, however, not quantified. No diastereomers of mitragynine were present in the prepared calibration samples. Thus, the presence of mitragynine diastereomers in real samples is not an artefact of the employed sample preparation procedure.

4.3. Ethanol determination

Average blood alcohol concentrations were 0.00‰ and 0.01‰ for cases 1 and 2, respectively.

5. Discussion

5.1. Case 1

The primary cause of death in subject 1 was the aspiration of chyme.

The quantitative analysis of mitragynine pointed towards the consumption of a high amount of kratom close to death, although postmortem redistribution of mitragynine cannot be fully excluded. However, in a study of postmortem concentrations in a mitragynine related fatality three days after death, McIntyre *et al.* [44] concluded that the substance is unlikely to be prone to postmortem redistribution. Blood collection from our subject took place two days after death.

Nonetheless, despite an extremely high concentration of mitragynine detected in the femoral blood, a cause of death other than an acute mitragynine overdose could be derived for subject 1. Urinary retention points towards a loss of consciousness, which can be explained by the results obtained during the toxicological analyses. The benzodiazepine analogue, etizolam, in femoral blood is in a concentration range that is likely to result in toxic effects.

Table 2
Relevant substances detected in femoral blood and urine of case 2, together with the lowest known postmortem blood concentrations in fatalities for each substance [45]. With the exception of mitragynine and GHB, drug concentrations in urine were not determined. (MDA = 3,4-methylenedioxy-*N*-amphetamine; MDMA = 3,4,methylenedioxy-*N*-methylamphetamine; 6-MAM = 6-*O*-monoacetylmorphine; GHB = γ-hydroxybutyric acid).

Case 2			
Femoral blood			Urine
Substance	Concentration detected	Lowest known postmortem concentrations in fatalities	Substance
Mitragynine	10 μg/L	Unknown	Mitragynine (<10 μg/L)
Mitragynine diastereomers	Not quantified	–	Mitragynine diastereomers
Amphetamine	34 μg/L	500 μg/L	Amphetamine & metabolites
MDA	40 μg/L	1800 μg/L	–
Methamphetamine	3300 μg/L	90 μg/L	Methamphetamine & metabolite
MDMA	1400 μg/L	500 μg/L	MDMA & metabolites
Pseudoephedrine	8 μg/L	–	(Pseudo-)ephedrine
Morphine	210 μg/L	10 μg/L	Morphine
6-MAM	41 μg/L	–	–
Paracetamol	1.9 mg/L	90 mg/L	Paracetamol
Codeine	24 μg/L	–	Codeine
–	–		Caffeine
GHB	480 mg/L	103 mg/L	GHB (160 mg/L)

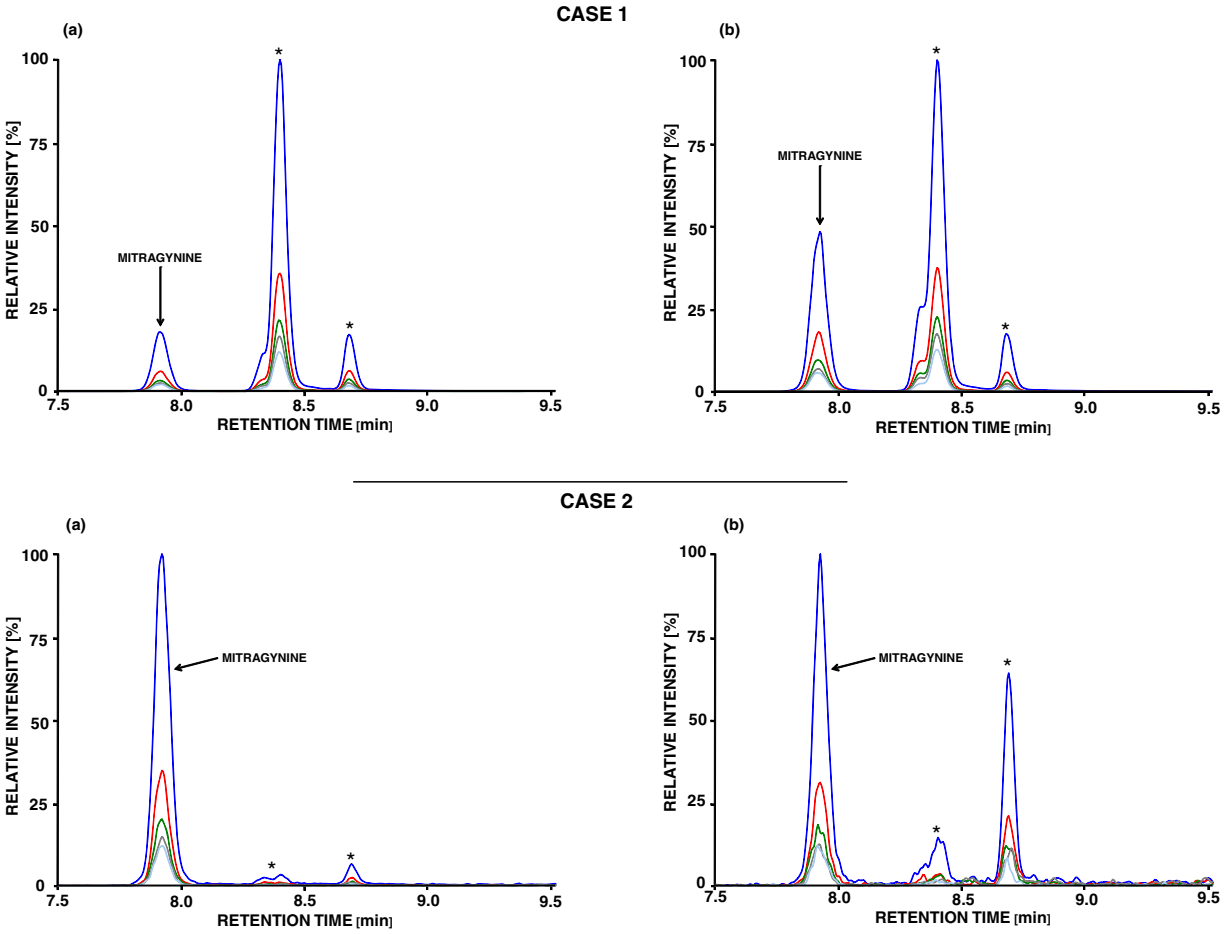

Fig. 3. Chromatograms showing the differences in peak intensity between mitragynine and its diastereomers for the (a) blood and (b) urine samples of cases 1 and 2. The peaks indicated with asterisks (*) are likely to present mitragynine diastereomers. SRM transitions: 399.2/174.0; 399.2/159.0; 399.2/130.1; 399.2/115.1 and 399.2/75.0.

With the exception of pregabaline, of which the levels were within therapeutic range, all other substances in the femoral blood of subject 1 were below therapeutic levels [45,46]. Thus, the levels of mitragynine, especially in combination with those of etizolam, are likely to have caused the subject to fall unconscious shortly before his death. The aspiration of chyme as a result of such a loss of consciousness could be confirmed by the pathological findings during the autopsy, which finally led to the fatality.

The sedative effects of mitragynine are not only in accordance with its expected concentration dependent properties, but are also very particular for the type of kratom found at the scene of death. High concentrations of mitragynine are known to cause opioid-like effects, including sedation [15]. “Red Vein” is also known to show extremely strong sedative effects [32,33] during the so-called second time zone, a phase which, according to the distributors of kratom, follows an initial stimulating effect [48,49]. This further underlines the finding of the fractured arm, which, as discussed in the case history, was the result of the subject's fall from a window. The sedation caused by kratom and also by the other substances with similar side effects, likely played a role in this incident. Furthermore, it is to be expected that a fractured bone would be associated with immense pain. Yet the subject refused medical treatment. It is likely that this pain was alleviated by the activity of mitragynine on the opioid receptors, which would explain a lack of urgency for medical help.

5.2. Case 2

Subject 2 died as a result of a mixed intoxication with heroin, methamphetamine, MDMA and GHB, although it cannot fully be excluded that the high GHB concentrations may at least partially have resulted from postmortem decomposition. The detection of morphine and codeine in a ratio of approximately 10:1, together with the detection of 6-MAM in blood confirm the consumption of heroin by the subject shortly before his death. Also the subtherapeutic concentration of paracetamol suggests the use of this substance as cutting agent for heroin. Morphine as metabolite of heroin and MDMA were both in a range of previously reported fatalities as direct result of these substances.

In contrast to case 1, much lower concentrations of mitragynine were detected in the biological fluids of subject 2. In view of the fact that mitragynine is a methyl ester type compound and that the corpse showed signs of decomposition, it cannot be ruled out that mitragynine was degraded postmortal to a significant extent. The particular strain of kratom found at the scene was not identified.

In conclusion, mitragynine was, at the most, merely a contributing factor to the death of subject 2.

5.3. Diastereomers of mitragynine

The identification of mitragynine diastereomers in blood and urine serves as marker for the consumption of kratom [43,50]. A clear stereoselective separation could be achieved in our chromatographical analysis. The ion ratios of the peaks following the

elution of mitragynine (indicated with asterisks in Fig. 3) strongly indicate mitragynine diastereomers. A more definite identification would require more detailed mass transition analyses [41,50,51]. This was, however not the main focus of this publication, but rather the concentrations of mitragynine itself.

An interesting observation when comparing the two chromatograms of our two cases is the remarkable differences in ratio of mitragynine to its diastereomers in both blood and urine samples. We, however, refrain from indicating defined diastereomer concentrations, because we cannot confirm that the MS/MS sensitivity of the diastereomers is in the same range as that of mitragynine. Nevertheless, as can be seen in Fig. 3, it is obvious that the ratio of diastereomers to mitragynine in case 1 is several fold higher as compared to that for case 2. In searching the literature for other blood concentrations of mitragynine and its diastereomers, we found at least two other cases with a higher diastereomer content compared to mitragynine [42,52]. At present we do not have a valid explanation for this difference in mitragynine/diastereomer signal intensity ratios. Factors such as variations in mitragynine content between different kratom products (e.g. due to the age of the plant), varying inter-individual pharmacokinetics, varying pharmacokinetic behavior between the diastereomers and even an enzymatic inversion of mitragynine to its antipodes after consumption might likely play a role. These suggestions at this point remain speculative and require further investigation.

Irrespective of the origin of these variations, it should still be considered that the pharmacological effects of kratom could be influenced by the ratios of mitragynine to its stereoisomers, but also to other minor alkaloids. In our study, we chose not to consider the presence of the less abundant, yet more potent 7-hydroxymitragynine. Our main focus with this publication was to add to postmortem mitragynine concentrations in fatalities related to the recreational use of kratom. More detailed investigations in this context are warranted.

6. Conclusion

In this publication we report two deaths related to the recreational use of kratom. In one of these two cases, the low mitragynine concentrations were at most a contributing factor to the fatality. In the second case, on the other hand, we report one of the highest blood and urine mitragynine concentrations published to date. Interestingly, these high concentrations were rather an indirect cause of death and both the high concentrations of mitragynine and the particular strain, i.e. red vein kratom, were likely to be contributing factors to the generated sedative effects.

Our results support the idea that mitragynine does not necessarily show high direct toxic or lethal potential on its own, even when taken in very high doses.

More detailed investigations into the toxicity of mitragynine, its chemical stability under postmortal conditions, as well as the exact mechanisms and constitution of the different mitragynine alkaloids, both in the plant and after metabolism, are required to allow for more valid interpretations of the role of kratom use in fatal cases.

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