Case Report

Unintentional Fatal Intoxications with Mitragynine and *O*-Desmethyltramadol from the Herbal Blend Krypton

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Abstract

The leaves of Kratom, a medicinal plant in Southeast Asia, have been used as an herbal drug for a long time. At least one of the alkaloids present in Kratom, mitragynine, is a mu-receptor agonist. Both Kratom and an additional preparation called Krypton are available via the internet. It seems to consist of powdered Kratom leaves with another mu-receptor agonist, *O*-desmethyltramadol, added. *O*-Desmethyltramadol is an active metabolite of tramadol, a commonly prescribed analgesic. We present nine cases of intoxication, occurring in a period of less than one year, where both mitragynine and *O*-desmethyltramadol were detected in the postmortem blood samples. Neither tramadol nor *N*-desmethyltramadol was present in these samples, which implies that the ingested drug was *O*-desmethyltramadol. The blood concentrations of mitragynine, determined by ultra-performance liquid chromatography–tandem mass spectrometry, ranged from 0.02 to 0.18 µg/g, and *O*-desmethyltramadol concentrations, determined by gas chromatography with nitrogen-specific detection, ranged from 0.4 to 4.3 µg/g. We believe that the addition of the potent mu-receptor agonist *O*-desmethyltramadol to powdered leaves from Kratom contributed to the unintentional death of the nine cases presented and conclude that intake of Krypton is not as harmless as it often is described on internet websites.

Introduction

Herbal drugs have always been popular because of their natural origin, and they are often thought of as safe alternatives to synthetic drugs. However, in recent years, several preparations sold as herbal drugs have actually been plant material spiked with synthetic active components. The most well-known series of preparations is Spice, which still is frequently used and which contains synthetic cannabinoid receptor agonists added to the dried leaves of plant material with little or no pharmacological effect (1–3).

Another example is the leaves of the medicinal plant *Mitragyna speciosa*, known as Kratom, which is native to Southeast Asia and has traditionally been used as a herbal drug for various indications (4–8). In low doses, it has a stimulant effect, and there are sedative and opioid-like effects after high doses (4). Even if more than 25 different alkaloids have been identified in this plant, the major constituent is mitragynine, which also is responsible for the opioid effects through the mu-receptor (9,10). Mitragynine has several diastereoisomers that also are present in various concentrations depending on the age and origin of the plant (9,10).

Currently, Kratom can easily be bought via the internet and is used worldwide. Even though Kratom has its own opioid effect, it is also available with another mu-agonist added. The preparation, called Krypton, consists of powdered Kratom leaves mixed with *O*-desmethyltramadol, the active metabolite of the commonly used analgetic tramadol (2,11). Tramadol is metabolized through O- and N-demethylation via cytochrome P-450 iso-enzymes. *O*-Desmethyltramadol, the only metabolite with pharmacological activity, has a higher affinity for the mu-receptor than tramadol itself (12). Although tramadol is considered a safe drug, there is an abuse potential and unintentional fatalities with tramadol have been described (13,14) even though they seem to be scarce. However, reports of its detection in autopsy cases and in the living confirm that there is an abuse potential (15,16).

The addition of another mu-receptor agonist makes Krypton more powerful than the leaves of Kratom alone. In addition, Krypton is sold in large quantities in containers with more than 50 g even though the dose recommended at the websites is as small as 0.5 g. The risk for unintentional overdose is

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therefore high. Since November 2009, we have identified nine cases of fatal overdose in which postmortem blood samples were positive for both mitragynine and *O*-desmethyltramadol without the parent drug. We believe that the intake of Krypton was the cause of these deaths and describe the circumstances in this paper.

Materials and Methods

Medicolegal autopsies are performed at six departments of forensic medicine in Sweden. At autopsy, samples of femoral blood, urine, and vitreous humor are routinely collected and submitted for toxicology screening to one central laboratory, the Department of Forensic Genetics and Forensic Toxicology in Linköping. Other samples, such as heart blood, muscle, hair, etc., are collected if routine samples are not available or if otherwise necessary. Potassium fluoride (1–2%) is added to the samples as a preservative. Mitragynine was obtained from ChromaDex (Irvine, CA), and LSD-d_3 was obtained from Cerilliant (Round Rock, TX). Speciogynine, speciociliatine, and mitraciliatine for identification of these mitragynine diastereoisomers were kind gifts from Professor Hans H. Maurer, Homburg, Germany.

Routine toxicological analyses

From more than 95% of the annual ~5000 medicolegal autopsies, samples were analyzed considering pharmaceuticals, including tramadol and metabolites. Alcohols and acetone were analyzed with headspace gas chromatography (GC) according to a previously described method (17). Narcotic drugs were analyzed in urine or blood using immunoassays and positive indications confirmed in blood with GC–mass spectrometry (MS) or liquid chromatography (LC)–tandem MS. About 150 different pharmaceutical drugs were analyzed by GC with nitrogen-specific detection (NPD), using an Agilent 5890 with original nitrogen-phosphorus detector and an Agilent 6890 with Blos bead detector. Two GC columns with different polarity, DB5 and DB17, were used to improve the identification of the analytical findings. The blood samples were extracted alkaline and neutral with butyl acetate (18). Tramadol and *O*-desmethyltramadol, included in this determination of phar-

maceuticals, were extracted alkaline. Briefly described; 1.0 g blood was extracted with 0.4 mL buthyl acetate after adding 0.3 mL Tris-buffer (1 M, pH 11) and 30 µL internal standard (0.05 mg cyclizine). After extraction and centrifugation, an aliquot was analyzed with GC–NPD. Calibration curves for tramadol ranged from 0.05 to 5 µg/g blood and for *O*-desmethyltramadol from 0.1 to 5 µg/g blood. Calibrators and controls were made by adding standard solutions to drug-free blood. Long-term interday imprecision and accuracy for *O*-desmethyltramadol and tramadol are presented in Table I. *N*-Desmethyltramadol was also included in the analysis, although only qualitatively.

Analysis of mitragynine

Postmortem blood was prepared using liquid–liquid extraction with ethyl acetate under basic conditions. To 1.0 g blood,
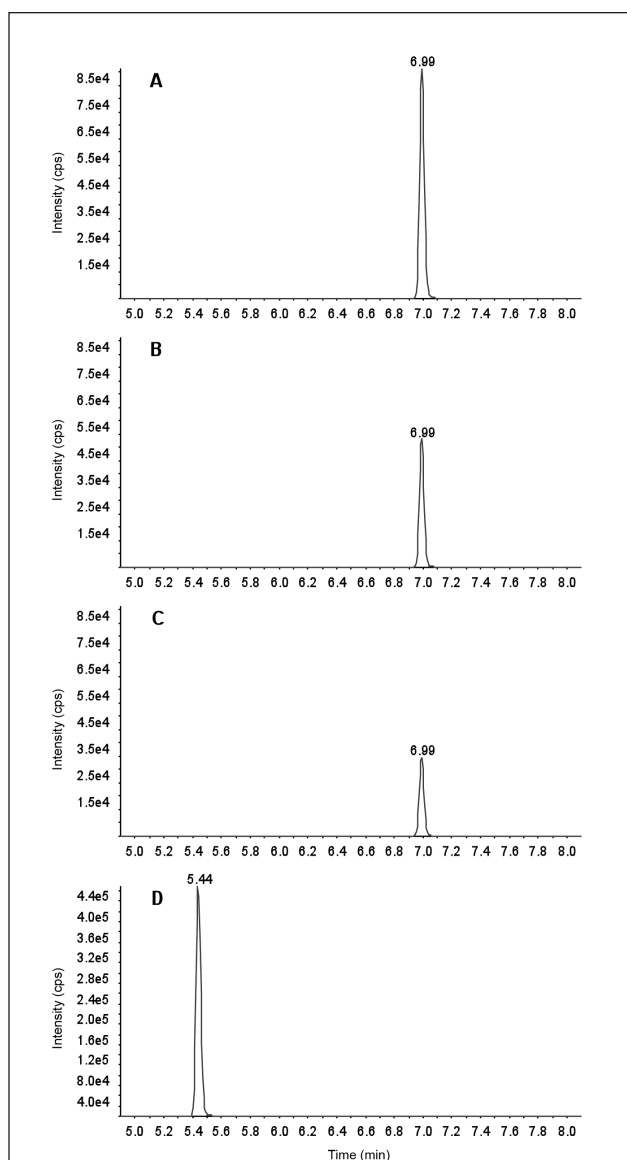

Figure 1. Chromatograms from a low quality control 0.01 µg mitragynine/g transitions 399/174 (A), 399/226 (B), and 399/159 (C) and internal standard, LSD-d_3, transition 327/226 (D).

Table I. Long-Term Interday Imprecision and Accuracy for *O*-Desmethyltramadol and Tramadol in Blood

	N	Mean (µg/g)	CV (%)	Accuracy (%)
O-Desmethyltramadol				
Low 0.2 µg/g	183	0.22	14.7	110
High 1.0 µg/g	184	1.08	13.2	108
Tramadol				
Low 0.1 µg/g	184	0.10	6.0	100
High 3.0 µg/g	185	3.08	5.2	103

25 ng of LSD-d$_3$ was added as internal standard. After addition of 0.5 mL Tris-buffer (1 M, pH 11) and 3 mL ethyl acetate, the sample was shaken for 15 min and then centrifuged; the organic phase was evaporated and then reconstituted in a 50:50 mixture of mobile phases A and B. The LC–MS–MS system
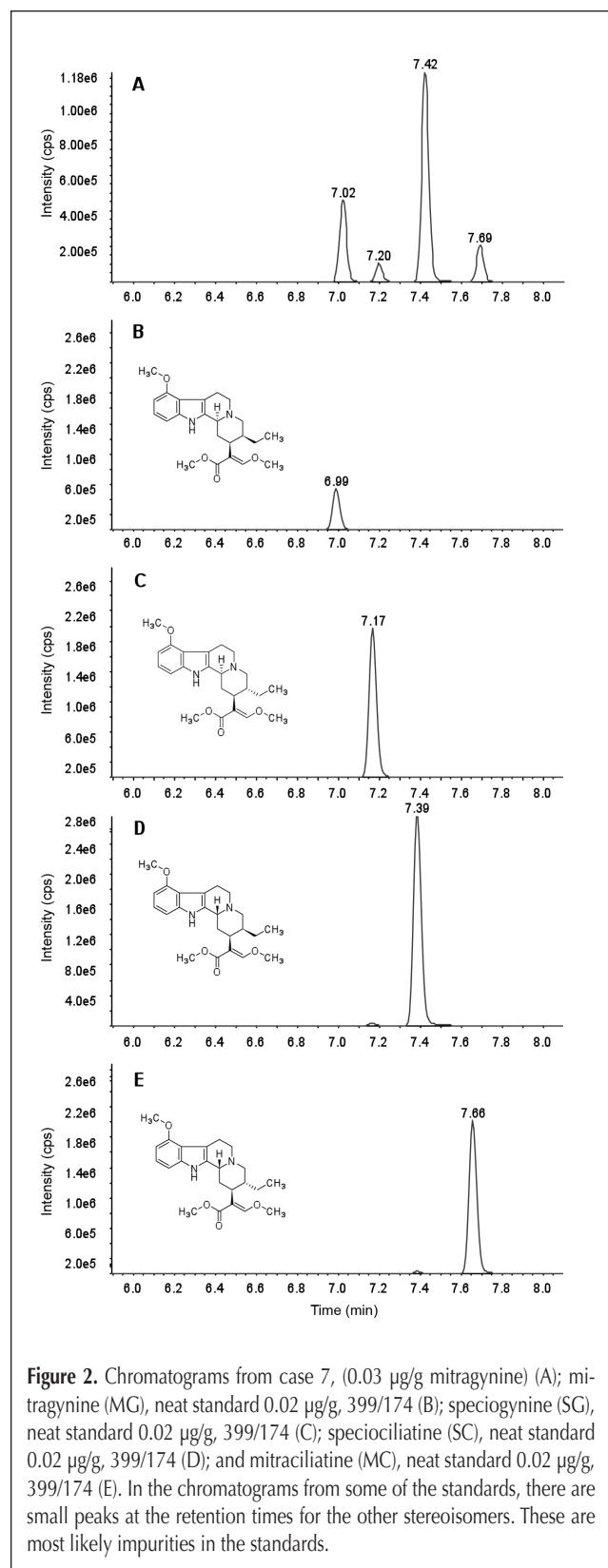
consisted of a Waters ACQUITY UPLC® (ultra-performance LC) with a Binary Solvent Manager, Sample Manager, and Column Manager (Waters, Milford, MA) connected to an API 4000™ triple-quadrupole instrument (AB SCIEX, Stockholm, Sweden) equipped with an electrospray interface (TURBO V™ source, TurboIonSpray® probe) operating in the multiple reaction monitoring (MRM) mode. Ion spray voltage was set to 4500 V. Nitrogen was used as the nebulizer gas (345 kPa), heater gas (517 kPa at 500°C), curtain gas (207 kPa), and as collision-activated dissociation gas (set on 5). UPLC was performed using an ACQUITY UPLC high-strength silica (HSS) T3 column (1.8 µm, 150 × 2.1 mm, Waters), preceded by a 0.2-µm column filter (Waters), and operated at 0.5 mL/min with a total run time of 12 min. Mobile phase A consisted of 0.05% formic acid in 10 mM ammonium formiate, and phase B was 0.05% formic acid in acetonitrile. The chromatographic system was run in a linear gradient from 1 to 50% phase B in 8 min, then increased to 95% phase B in 2 min, held at 95% phase B for 1 min, followed by a 0.9 min equilibration with 99% phase A. The injection volume was 1 µL, and the Column Manager temperature was set to 60°C. Instrument control, integration, and calculation were performed using Analyst™ 1.4.2 software. Quadratic regression analysis with 1/x weighting was used for the calibration curves. The final MRM method included three transitions 399/174, 399/226, and 399/159 for mitragynine and 327/226 for the internal standard, LSD-d$_3$,
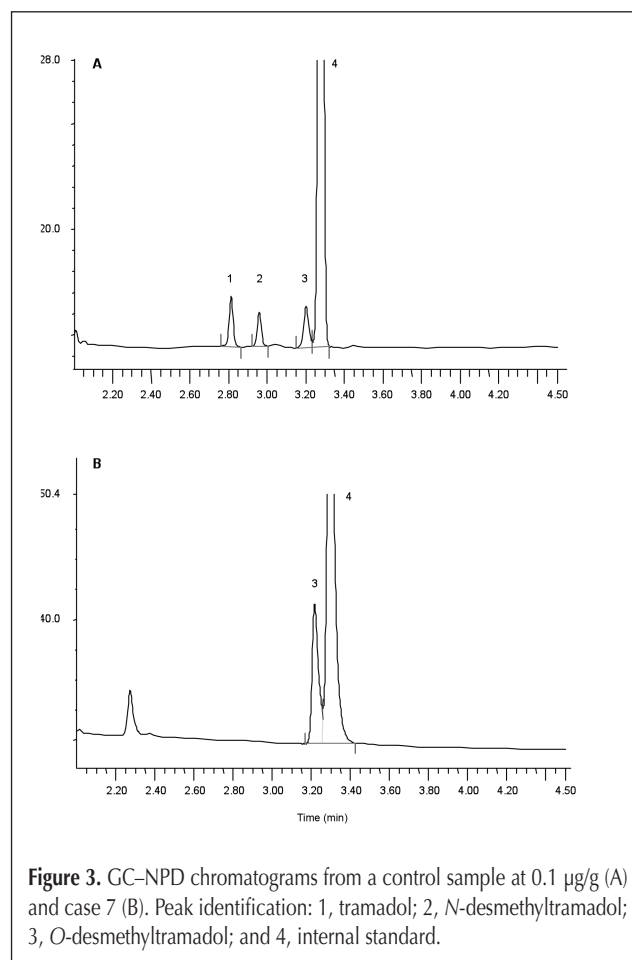

Figure 2. Chromatograms from case 7, (0.03 µg/g mitragynine) (A); mitragynine (MG), neat standard 0.02 µg/g, 399/174 (B); speciogynine (SG), neat standard 0.02 µg/g, 399/174 (C); speciociliatine (SC), neat standard 0.02 µg/g, 399/174 (D); and mitraciliatine (MC), neat standard 0.02 µg/g, 399/174 (E). In the chromatograms from some of the standards, there are small peaks at the retention times for the other stereoisomers. These are most likely impurities in the standards.

Figure 3. GC–NPD chromatograms from a control sample at 0.1 µg/g (A) and case 7 (B). Peak identification: 1, tramadol; 2, N-desmethyltramadol; 3, O-desmethyltramadol; and 4, internal standard.

Table II. Toxicology and Demographic Data from Nine Cases of Accidental Drug Poisoning*

Case	Age/Sex (years)	Lung Weight (Right/Left) (g)	O-DMT[†] (µg/g)	Mitragynine (µg/g)	Other Drugs in Blood (µg/g)	Significant Autopsy Findings	Circumstances
1	22/male	R 828 L 732	0.4	0.07	0.14 alprazolam 0.09 ethanol[‡]	Congestion of lungs	Found dead at home. Previous history of drug abuse. Ordered Krypton via the Internet, probably for the first time.
2	35/male	R 804 L 722	0.7	0.16	0.3 alimemazine 0.1 DMA 0.7 venlafaxine 0.1 O-DMV	Edema and congestion of lungs	Found dead in mother's home. Previous history of drug abuse
3	30/female	R 625 L 590	0.5	0.04	0.6 fluoxetine 0.5 norfluoxetine 19.8 phenazon 0.2 olanzapine 0.3 diazepam 0.3 nordiazepam 5.0 pregabalin 0.04 amphetamine	Congestion of lungs Liver steatosis	Found dead at home. Previous history of drug abuse.
4	33/male	R 831 L 668	1.5	0.05	0.2 alimemazine 0.2 DMA 0.1 olanzapine 0.05 nordiazepam 0.002 THC	Edema and congestion of lungs Hepatitis Liver steatosis Mb Hodgkin Aspiration of stomach contents	Found dead in a friend's bedroom. Took Krypton, then fell asleep. Previous history of drug abuse.
5	27/male	R 695 L 640	4.3	0.18	0.2 alimemazine 0.1 mirtazapine 0.1 venlafaxine 0.09 diazepam 0.2 nordiazepam 0.0004 buprenorphine	Brain edema Lung edema	Found dead at home. Previous history of drug abuse.
6	27/male	R 712 L 690	1.2	0.05	0.04 zopiclone 0.01 ethanol[‡]	Brain edema Lung edema	Found dead at home. Ordered Krypton via internet. Previous history of drug abuse.
7	24/male	R + L 1456	1.1	0.03	0.14 alprazolam 0.20 amphetamine 0.0006 THC	Brain edema Congestion of lungs	Found dead in friend's home. Previous history of drug abuse.
8	25/female	R 620 L 410	0.8	0.02	1.0 venlafaxine 1.1 O-DMV 0.06 zopiclone	Congestion of lungs	Admitted to hospital unconscious with asystole 2 h after drinking tea made from Krypton.
9	32/male	R 848 L 770	1.1	0.05	0.8 citalopram 0.07 alprazolam 0.007 THC	Brain edema Lung edema	Found dead at home. Previous history of drug and alcohol abuse.

* The cause and manner of death was accidental drug intoxication in all cases.
† Abbreviations: O-DMT, O-desmethyltramadol; DMA, desmethylalimemazine; O-DMV, O-desmethylvenlafaxine; and THC, tetrahydrocannabinol.
‡ Ethanol reported at g/dL.

with a dwell time of 75 ms for each transition. Criteria for identification were based on a qualifier ratio within 30% of the target ratio.

Method characteristics for the analysis of mitragynine

Matrix effects were evaluated using post-column infusion of mitragynine together with the injection of 10 different post-mortem blood samples negative for mitragynine. Working range was verified by analysis of triplicates at seven concentrations from 0.008 to 0.20 µg/g blood. The within-day ($n = 5$) imprecision was estimated by analyzing five replicates at two concentrations, 0.01 and 0.15 µg/g blood. Calibrators and controls were made by adding standard solutions to drug-free blood. Final calibration concentrations were 0.008, 0.01, 0.05, 0.08, 0.10, 0.15, and 0.20 µg/g blood.

Identification of mitragynine diastereoisomers

Speciogynine, speciociliatine, and mitraciliatine, all three diastereoisomers of mitragynine, have been identified in urine samples from both humans and dosed rats (9,10). In the blood samples, we observed a cluster of four peaks with the same transitions. Therefore, we aimed at identifying these potential isomers. Each standard was analyzed separately and compared with the retention times of those in the peak cluster from the authentic samples.

Cases

The first case appeared in November 2009 and was recognized because of the presence of *O*-desmethyltramadol in the absence of the parent drug tramadol. Indeed, this was an unusual finding, even though tramadol itself is very commonly found in autopsy cases (19). Because of the reports available about Krypton, we analyzed and detected mitragynine in both blood and urine. During the spring 2010, several more cases were observed, and the current method for mitragynine, using a 150-mm column with a less-steep gradient was developed to increase the separation between the diastereoisomers. Also, LSD-d_3 was added as internal standard. Thus, the cases we present were not analyzed with the same chromatographic method for mitragynine.

Results

The LC–MS–MS method showed no matrix effects when investigating 10 different postmortem blood samples. The within-day imprecision of the mitragynine quantitation was 2.4% and 4.3% at the low and high level. A linear calibration model resulted in a variation less than 8% at the 7 levels, as well as accuracy between 97 and 102%. Chromatograms of a control sample at 0.1 µg/g are shown in Figure 1, and chromatograms from case 7 and the mitragynine diastereoisomers are shown in Figure 2. Figure 3 shows the GC–NPD chromatograms from case 7 and a control sample containing tramadol and its two

demethylated metabolites. *O*-Desmethyltramadol precision data showed good performance over time as seen in Table I. The cases and their toxicological results are described in Table II. The autopsy findings were non-specific; in most cases, brain and lung edema and congestion of inner organs were noted. All blood samples, except case 3, were femoral blood. In summary, none of the cases presented with tramadol in blood, indicating that *O*-desmethyltramadol is not present in these cases as a metabolite but indeed was the ingested drug. This is further supported by the presence of mitragynine because they appear together in Krypton preparations. Several other psychotropic drugs were detected in each victim and could have contributed to death. The concentrations of *O*-desmethyltramadol ranged between 0.4 and 4.3 µg/g, and those of mitragynine ranged between 0.02 and 0.18 µg/g. The mitragynine diastereoisomers were identified in all cases.

Discussion

One of the most important conclusions from the present study is that the use of legal herb preparations might be associated with a high risk. In fact, the user has very little control over the contents of powders and plant material bought through the internet, because they might be spiked with one or more powerful synthetic drugs (1,3,20). A recent report on unintentional fatal intoxications with medications containing tramadol showed that only 17 cases were identified in Sweden over a 10-year period (14). However, the present study shows that over a period of less than one year, nine cases of poisoning with *O*-desmethyltramadol emerged. The potency of *O*-desmethyltramadol has been described as twice that of the parent compound tramadol (16). Femoral blood concentrations of tramadol higher than 1.0 µg/g are considered toxic and possibly fatal (14). Considering the higher potency of *O*-desmethyltramadol, the concentrations in the reported cases seem to be in the high range, suggesting overdose. The fact that Krypton is sold in packages containing large quantities even though the recommended dose is only 0.5 g supports this. The finding of heavy lungs in all cases but one also points towards respiratory depression and opiate overdose or a combination of *O*-desmethyltramadol and other drugs.

The absence of tramadol strongly suggests that the ingested drug was *O*-desmethyltramadol. During treatment, tramadol steady-state levels in blood are always higher than those for *O*-desmethyltramadol. Even though *O*-desmethyltramadol has a slightly longer half-life than tramadol, 7.4 h versus 6.3 h (16), detection of the metabolite only in the blood after tramadol use is unusual. The absence of *N*-desmethyltramadol is additional support for the ingestion of *O*-desmethyltramadol in the presented cases (Figure 3).

The contribution of mitragynine including its isomers in these cases is unclear because no reference data on blood concentrations are yet available. However, its agonist effects on the mu-receptor suggest that it may have contributed to the deaths. In addition, other alkaloids in Kratom have been investigated for their mu-receptor agonist properties, and 7-hy-

droxymitragynine was found to have 30 times higher affinity than mitragynine in in vitro experiments as well as in an animal model (5,21,22).

Conclusions

We believe that the addition of the potent mu-receptor agonist *O*-desmethyltramadol to powdered leaves from Kratom contributed to the unintentional death of the nine cases presented. We conclude that intake of the herbal blend Krypton is not as harmless as it often is described on internet websites, and the large packages sold increase the risk for unintentional overdose.

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