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ORIGINAL ARTICLE

Suspected Adulteration of Commercial Kratom Products with 7-Hydroxymitragynine

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Abstract
Introduction Kratom (*Mitragyna speciosa*), a plant native to Southeast Asia, has been used for centuries for its stimulant and opium-like effects. Mitragynine and 7-hydroxymitragynine, exclusive to *M. speciosa*, are the alkaloids primary responsible for Kratom's biologic and psychoactive profile, and likely contribute to its problematic use. We purchased several commercially available Kratom analogs for analysis and through our results, present evidence of probable adulteration with the highly potent and addictive plant alkaloid, 7-hydroxymitragynine.
Methods A simple and sensitive LC-MS/MS method was developed for simultaneous quantification of mitragynine and 7-hydroxymitragynine in methanol extract of marketed Kratom supplements.
Results We found multiple commercial Kratom products to have concentrations of 7-hydroxymitragynine that are substantially higher than those found in raw *M. speciosa* leaves.
Conclusions We have found multiple packaged commercial Kratom products likely to contain artificially elevated concentrations of 7-hydroxymitragynine, the alkaloid responsible for *M. speciosa's* concerning mechanistic and side effect profile. This study describes a unique form of product adulteration, which stresses the importance of increased dietary supplement oversight of Kratom-containing supplements.

Keywords Kratom · 7-Hydroxymitragynine · Mitragynine · *Mitragyna speciosa* · Drugs of abuse

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Background

Kratom (*Mitragyna speciosa*), a member of the coffee family, is indigenous to Southeast Asia. Kratom has been utilized for thousands of years to enhance work productivity, cultural ceremonies, for medicinal purposes, and as a substitute for ethanol or opium [1–4]. Kratom contains a multitude of alkaloids, many of which are responsible for the biological effects of the plant [5]. Despite being criminalized in foreign countries with punishments of up to 70 years or more [1, 6], Kratom use has become increasingly popular in the USA, where it is still legal to possess and consume in most states [7–11].

M. speciosa contains over 40 different alkaloids which comprise 0.5–1.5 % of the plant matter [12, 13]. Of these, the most clinically consequential are mitragynine and 7-hydroxymitragynine, compounds which are found exclusively in *M. speciosa* [6, 14, 15]. Mitragynine, a major indole-containing constituent similar in structure to yohimbine [13], accounts for up to 66 % of the plant's alkaloid content [15, 16]. It produces opioid-like effects predominantly via mu- and delta-opioid receptor agonism [5, 13, 17] in addition to modulation of the descending serotonergic and noradrenergic

pathways [18]. In vitro characterization of mitragynine receptor binding in the central nervous system reveals the complexity with which Kratom acts (see Table 1) [10]. In regard to its antinociceptive actions, mitragynine is one third as potent as morphine and three times as potent as codeine [18, 19].

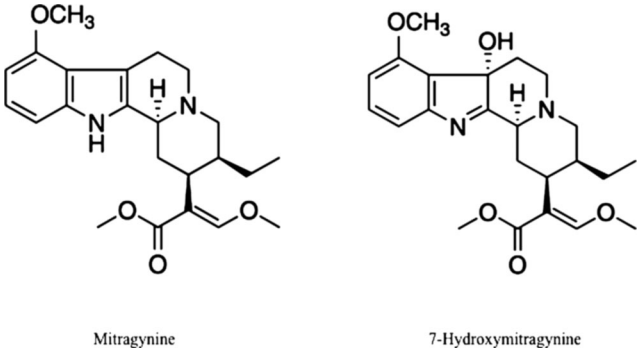
A minor alkaloid constituent of Kratom, 7-hydroxymitragynine, was first described in 1994 and is structurally identical to mitragynine except the addition of a hydroxyl group at the C7 position (see Fig. 1) [16]. Because auto-oxidation of mitragynine into 7-hydroxymitragynine occurs [16], this minor constituent may be an innate natural product or arise from mitragynine metabolism within the *M. speciosa* plant. Accounting for roughly 2 % of the plant's alkaloid content [16], 7-hydroxymitragynine is an opioid receptor agonist (like mitragynine) but demonstrates potent mu and kappa receptor selectivity [12]. This alkaloid is the major contributing factor for Kratom's analgesic properties, demonstrating opioid receptor affinity up to 17 times that of morphine [20–22]. It may also contribute to problematic Kratom use, which has been reported numerous times in the literature [10, 23–26]. 7-Hydroxymitragynine's role in Kratom abuse is supported by Matsumoto et al.'s findings (2005), which demonstrate development of tolerance, cross-tolerance to morphine, and physical dependence in 7-hydroxymitragynine treated mice [27]. Well established is the knowledge that morphine tolerance and physical dependence are secondary to mu-opioid receptor agonism [28, 29]. Therefore, 7-hydroxymitragynine, a potent mu-opioid receptor agonist, is likely to be a major contributing factor to the addictive potential of Kratom.

We purchased several commercially available Kratom analogs for analysis and, through our results, present evidence of adulteration with the highly potent and addictive plant alkaloid, 7-hydroxymitragynine.

Table 1 Central nervous system receptor binding data for mitragynine [10]

Percentage inhibition of radioligand binding by mitragynine at selected receptor systems
Adenosine A2A 65.66
Adrenergic (alpha 2) 61.92
Dopamine D2s 54.22
Opioid, mu 89.52
Opioid, kappa 90.21
Opioid, delta 7.00
Serotonin, 5HT2C 58.77
Serotonin, 5HT7 64.41
Dissociation constants for opioid receptor binding
Mu receptor 204 ± 26 nM
Delta receptor 2250 ± 120 nM
Kappa receptor 455 ± 47 nM

For contextual purposes, the rank order of binding affinity at the mu opioid receptor from highest to lowest is as follows: fentanyl [108] > 7-hydroxymitragynine [20] > mitragynine [21] > morphine [21]



Mitragynine 7-Hydroxymitragynine

Fig. 1 Structures of mitragynine and 7-hydroxymitragynine

Methods

Materials and Reagents

Mitragynine and 7-hydroxymitragynine (purity ≥98 %; IP grade) as free base (purity >98 %) were synthesized in-house. Mitragynine was isolated from dried leaves of *M. speciosa* as described by Ponglux et al. [16]. Synthesis of 7-hydroxymitragynine was performed in-house as reported earlier by Takayama et al. and Ponglux et al. [16, 21]. Purities of mitragynine and 7-hydroxymitragynine were determined by 1H nuclear magnetic resonance (NMR), 13C NMR, high performance liquid chromatography, elemental analysis, and high-resolution mass spectrometry. Ondansetron (internal standard [IS]) as ondansetron hydrochloride dihydrate was procured from AChemTek Inc. (Worcester, MA, USA). LC-MS grade acetonitrile, methanol, water, and ammonium acetate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Kratom supplements as Phoria^{tm} (Miami, FL, USA) red, Phoria^{tm} green, Phoria^{tm} regular, Phoria^{tm} Borneo white vein, Phoria^{tm} Borneo red vein, Phoria^{tm} Borneo green vein, Phoria^{tm} maeng da blue lotus, Phoria^{tm} maeng da kava, Green vein extra strength Kratom shot (Orbit distributors, Houston, TX, USA), and Viva Zen (Vivazen distribution, Salt Lake City, UT, USA) were purchased from a local market.

Preparation of Standards and Quality Control Samples

The primary stock solutions (1 mg/mL) of mitragynine and 7-hydroxymitragynine were separately prepared by dissolving the requisite amount in acetonitrile. The primary stock solutions were further diluted with acetonitrile for the preparation of working stock solutions (WS) (100 μg/mL). Combined spiking stock solutions (CSSs) containing 10, 7.5, 4.5, 2.5, 1.25, 0.25, 0.125, and 0.05 μg/mL for calibration standards (CS) and 9, 5, 0.1

and 0.05 μg/mL for quality control (QC) standards of each analyte were prepared by appropriately diluting the WS in acetonitrile. The CS containing 1, 2.5, 5, 25, 50, 90, 150, and 200 ng/mL of mitragynine and 7-hydroxymitragynine were prepared by diluting 2 μL of the individual CSS to 100 μL with methanol. The QC standards (N = 6, each) were also prepared in replicates containing 1 ng/mL (lower limit of quantification [LLOQ]), 2 ng/mL (low quality control [LQC]), 100 ng/mL (medium quality control [MQC]), and 180 ng/mL (high quality control [HQC]) by the same dilution scheme. WS containing 100 μg/mL of the IS was prepared by appropriate dilution in acetonitrile. All the stocks (MS, WS, and CSS solutions) were stored at 4 °C and vortex mixed. The stability of stock solutions of mitragynine and 7-hydroxymitragynine was assessed by comparing analytical standards prepared in acetonitrile form stored stock solutions (6 months at 4 °C) with freshly prepared stock solutions. Both mitragynine and 7-hydroxymitragynine were found chemically stable in stock solutions and the variance between the two less than 10 %.

Equipment and Conditions

The analysis of mitragynine and 7-hydroxymitragynine was performed using acquity ultra-performance liquid chromatography (Waters, Milford, MA, USA) equipped with triple quadrupole Micromass Quattro Micro™ (Waters, Milford, MA, USA) detector. The standard electrospray ionization (ESI) in the positive mode using multiple reaction monitoring (MRM) using parent ion to daughter ion transitions from m/z 399.39 → 174.37, 415.34 → 190.4, and 294.37 → 170.40 with collision energy 32, 30, and 28 eV was employed for the analysis of mitragynine, 7-hydroxymitragynine, and IS, respectively. The cone voltage was set to 34, 30, and 28 V for mitragynine, 7-hydroxymitragynine, and IS, respectively. The source parameters, viz. capillary voltage, extractor voltage, RF lens, source temperature, desolvation temperature, cone gas flow, and desolvation gas, were 4 kV, 4 V, 0.2 V, 120 °C, 400 °C, 20 psi, and 800 psi, respectively. Dwell time for both analytes was set for 200 ms. Nitrogen was used as the nebulizer and cone gas, while argon was employed as collision gas.

A BEH C18 column (1.7 μm, 2.1 mm × 50 mm; Milford, MA, USA) was used. Optimum separation of the analytes was achieved by gradient elution started with pump A (0.1 % acetic acid in water) and pump B (0.1 % acetic acid in acetonitrile) supplying 90 and 10 % of the mobile phase components, respectively. The concentration of the mobile phase component in pump A decreased linearly to 60 % up to 5 min and kept constant up to 6 min followed by a linear increase to 90 % by 6.5 min and maintained up to 8.5 min. The flow rate of mobile phase was 0.2 mL/min. The temperature of peltier-tray was 10 °C, while column oven temperature was set to 30 °C. MassLynx software version 4.1 (Waters, Milford, MA, USA) was used for control of the equipment, data acquisition, and analysis.

Sample Preparation

The requisite amount of capsule formulations (Phoria[tm] red, Phoria[tm] green, Phoria[tm] regular, Phoria[tm] Borneo white vein, Phoria[tm] Borneo red vein, Phoria[tm] Borneo green vein, Phoria[tm] maeng da blue lotus, and Phoria[tm] maeng da kava) was weighted and soaked in methanol (IS; 10 ng/mL) for a concentration of 1 mg/mL. For liquid drinks, a 100 μL of formulation (Green vein extra strength Kratom shot and Viva Zen) was mixed up to 1 mL of methanol (IS; 10 ng/mL). The methanol extract was vortexed for exactly 10 min on BenchMixer (Benchmark, USA) at 2500 rpm. After vortex mixing, methanol extract was centrifuged at 13,000 rpm for 10 min. The supernatants (10 μL) were further diluted up to 1 mL with methanol (IS; 10 ng/mL). The methanol extracts were vortex mixed followed by centrifugation. The supernatants were injected onto the column for analysis.

Results

Product ion spectra and MRM chromatograms of mitragynine, 7-hydroxymitragynine, and IS are depicted in Figs. 2 and 3, respectively. As shown in Table 2, mitragynine was quantified as 9.7–19.0 μg/mg in capsule formulations; however, it was 190.7–396.4 ng/μL in liquid drinks. The 7-hydroxymitragynine was also present in all the methanol extracts of tested formulations for the concentration range of 93.0–593.2 ng/mg and 1.96–2.51 ng/μL in capsule and drink, respectively.

Discussion

We found multiple commercial Kratom products to have concentrations of 7-hydroxymitragynine that are substantially higher than those found in raw *M. speciosa* leaves [16, 27, 30]. Our findings strongly suggest adulteration of commercial Kratom products with 7-hydroxymitragynine, a plant alkaloid with potent mu-opioid receptor activity, a practice that increases the abuse liability and addictive potential of Kratom products. For example, concentrations of mitragynine and 7-hydroxymitragynine in raw *M. speciosa* leaves range between 23.6–24.0 μg/mg and 114–134 ng/mg, respectively [30]. Comparison of naturally occurring 7-hydroxymitragynine concentrations with the Phoria products listed in Table 2 demonstrates striking deviation from what would be expected. The

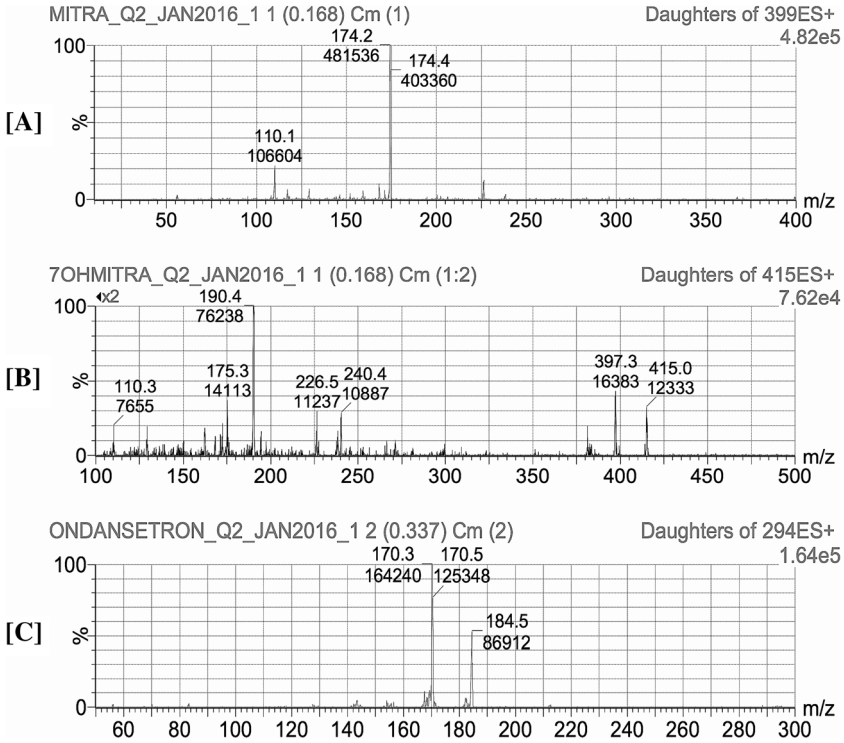


Fig. 2 Product ion spectra of **a** mitragynine (*m/z* 399.39), **b** 7-hydroxymitragynine (415.34), and **c** internal standard (*m/z* 294.37)

historically "minor" alkaloid constituent 7-hydroxymitragynine was found to be 109–520 % more concentrated in 7/8 capsule formulations studied. This research also highlights the need for regulations to prevent boosting the concentration of addictive

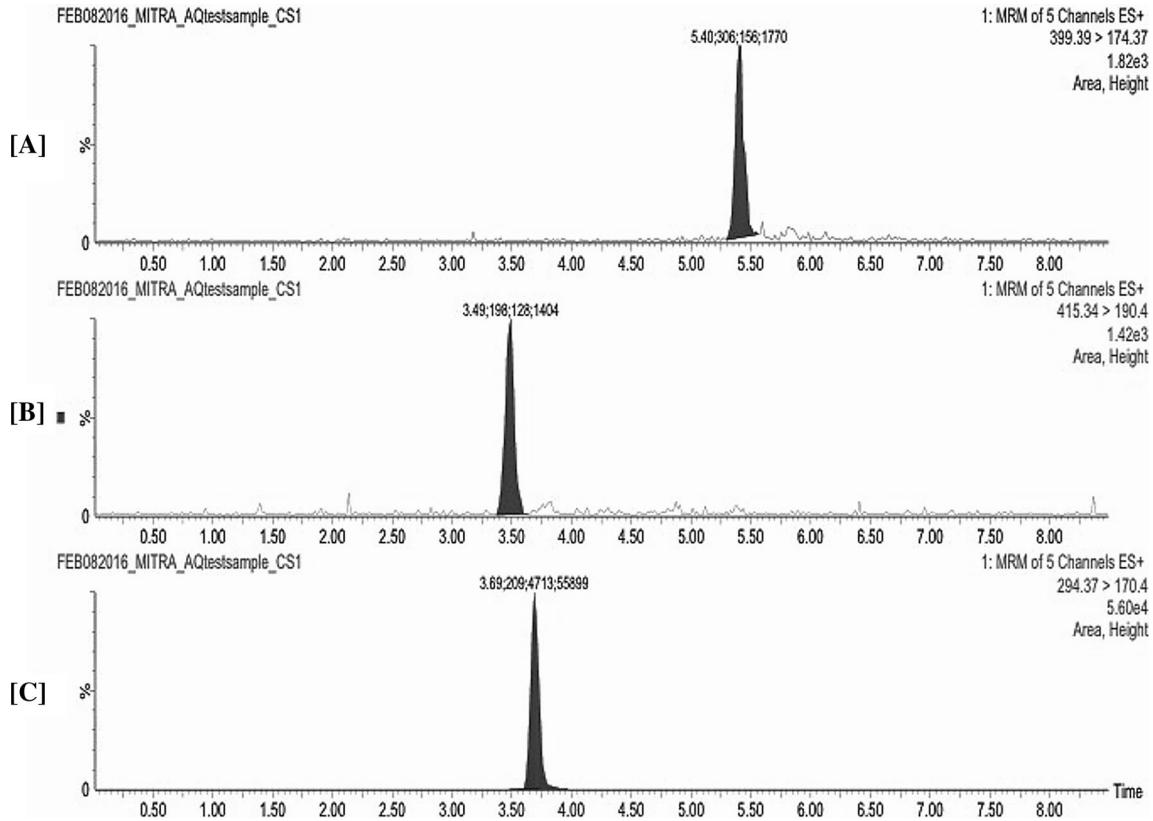


Fig. 3 Representative MRM chromatogram of **a** mitragynine (1.0 ng/mL; LLOQ), **b** 7-hydroxymitragynine (1.0 ng/mL; LLOQ), and **c** IS (10 ng/mL)

Table 2 Concentration of mitragynine and 7-hydroxymitragynine in naturally occurring Kratom leaf and marketed Kratom supplements

Brand name	Concentration	
	Mitragynine	7-Hydroxymitragynine
Natural Kratom leaf [30]	23.8 μg/mg (range 23.6–24.0)	124.0 ng/mg (114.0–134.0)
Phoria^{tm} Borneo white vein	18.3 μg/mg	593.2 ng/mg
Phoria^{tm} red	18.5 μg/mg	410.8 ng/mg
Phoria^{tm} green	11.7 μg/mg	378 ng/mg
Phoria^{tm} Borneo red vein	14.9 μg/mg	346.2 ng/mg
Phoria^{tm} maeng da kava	10.9 μg/mg	300.8 ng/mg
Phoria^{tm} maeng da blue lotus	9.7 μg/mg	146.7 ng/mg
Phoria^{tm} Borneo green vein	17.5 μg/mg	146.7 ng/mg
Phoria^{tm} regular	19.0 μg/mg	93.0 ng/mg
Kratom shot (liquid formulation)	190.7 ng/μL	2.51 ng/μL
Green vein extra strength (liquid formulation)	396.4 ng/μL	1.96 ng/μL

chemicals naturally occurring in Kratom, a unique form of supplement adulteration.

Kratom is sold on the internet and in specialty stores (i.e., head shops) as capsules, tablets, powder, concentrated extract, gum, or raw leaves for chewing or brewing tea [6, 13, 31]. Reported benefits include anticancer and antioxidant effects [32, 33], antiinflammatory properties [34], appetite reduction, glycemic control, treatment of chronic pain [35, 36], antidepressant actions [37], antidiarrheal and antimalarial properties [1, 38], treatment of ethanol [36, 39] or opioid withdrawal [1, 40, 41], and antibacterial activity [33]. Due to its stimulant and opioid-like properties, Kratom is also consumed for analgesic and recreational purposes [26].

Kratom produces dose-dependent clinical effects [3, 26, 42]. Historically, low doses of 1–5 g dried leaves produced stimulant effects which have been likened to those of cocaine [31]. At higher doses, opioid-like effects predominate [26]. In the past, low dose has been generally defined by users as 1–5 g of raw dried leaves and high dose as 5–15 g [11, 43]. As of 2014, however, the appearance of "super grade" strains of Kratom has redefined the amount of product needed to produce clinical effects. Low and high dosing are now considered by users to be 1–4 g and 4–8 g of dried leaf, respectively [43].

Adverse effects experienced by Kratom users are associated with chronicity of use. Short-term effects mimic those of standard opioid medications and include nausea, dizziness, constipation, itching, or sexual dysfunction [14, 44]. Chronic use results in insomnia, anorexia, weight loss, facial hyperpigmentation, dry mouth, polyuria, psychosis, or addiction [1, 45, 46]. Infrequent findings associated with chronic Kratom use include seizure or coma [10, 24, 47, 48], acute respiratory distress syndrome [49], hypothyroidism [50], intrahepatic cholestasis or toxic hepatitis [38, 48, 51], cardiotoxicity or dysrhythmia [52], and hypertension or nephrotoxicity [38]. Fatalities have been reported in the setting of therapeutic or supratherapeutic levels of coingestants such as sympathomimetics [53, 54], benzodiazepines and over the counter cold medications [55], antidepressants [56], muscle relaxers, and opioids [57, 58].

Supplement Regulation

Kratom is classified as a dietary supplement by the Federal Dietary Supplement Health and Education Act of 1994 and is defined as "a product intended for ingestion that contains a 'dietary ingredient' intended to add further nutritional value to (supplement) the diet" [59]. These dietary ingredients may include vitamins, minerals, amino acids, herbs or other botanicals or their concentrates, metabolites, constituents, or extracts [59]. Unlike FDA-approved drugs, laws surrounding dietary supplement regulation are lax. Adulteration is an exemplary repercussion of such laxity. Well documented are instances of diuretics, stimulants, anorectics, or oxidative uncouplers contained in weight loss supplements [60–74], steroids in performance enhancers [75–80], and phosphodiesterase 5 inhibitors contained within male enhancement products [72, 74, 81–93]. At least nine people died in Sweden following exposure to Kratom adulterated with *O*-desmethyltramadol, a potent opioid analgesic. All nine patients had pulmonary congestion and/or edema on autopsy, suggestive of respiratory depression [57]. Because pure Kratom does not appear to produce respiratory failure, these fatalities were likely due to *O*-desmethyltramadol alone and/or synergism with mitragynine or 7-hydroxymitragynine.

Legal Status

Thailand was the first country to criminalize Kratom under the Kratom Act of 1943 [6]. Today, Kratom is listed as a category V substance under the Thai Narcotics Act, similar

to cannabis and psychotropic mushrooms, with penalties of up to 1- or 2-year imprisonment for possession and production or disbursement of the substance, respectively [94, 95]. Since the Kratom Act, *M. speciosa* has been federally regulated in countries such as Denmark, Latvia, Lithuania, Poland, Romania, Sweden, Myanmar, Malaysia, Australia, and New Zealand [6]. In the USA, some cities and states have banned or are in the process of banning Kratom alkaloids due to health risks [96–102].

While Kratom is not yet regulated under the Controlled Substances Act in the USA, the federal government has steps to limit its use. The FDA, while classifying Kratom as a dietary supplement, also recognizes it as a "new dietary ingredient" as there is no evidence that it was sold as a supplement in the USA prior to October 15, 1994 [103]. A new dietary ingredient is only approved for marketing after a history of safe use or other evidence suggesting reasonable safety of the ingredient is documented [104]. Because Kratom has no evidence of reasonable safety, the FDA considers it adulterated and as a result has placed import bans on Kratom-containing supplements as of February 2014 [103, 105]. The FDA, with the help of US Marshals, has continued to seize Kratom-containing supplements on the grounds of product adulteration with an unapproved new dietary ingredient [106]. Additionally, the DEA has classified Kratom as a "drug and chemical of concern," given the lack of identified legitimate medical use and the potential risk to those who abuse it [107].

Limitations

Because 7-hydroxymitragynine is a product of mitragynine auto-oxidation, we considered whether the alkaloid is simply an artifact of testing procedures or of plant metabolism. As noted in our methods, stock solutions of mitragynine and 7-hydroxymitragynine are chemically stable over a period of 6 months. Likewise, no conversion of mitragynine into 7-hydroxymitragynine occurs in chloroform or passing through silica gel column chromatography; these findings suggest that 7-hydroxymitragynine is not an artifact of isolation [16]. The presence of normal mitragynine concentrations argues strongly that the observed supranormal 7-hydroxymitragynine concentrations arise from adulteration of the commercial products we analyzed, not variation in plant metabolism. We also recognize that it is possible, though unlikely, that the analyzed commercial Kratom strains are derived from *M. speciosa* plants with above average 7-hydroxymitragynine levels. While the alkaloid content of *M. speciosa* has been shown to vary based on geographic location and month of the year, the main indole alkaloid contents (i.e., mitragynine and 7-hydroxymitragynine) remain relatively stable compared to its oxindole counterparts, which show much greater variability [15]. To our knowledge, 7-hydroxymitragynine content has never been reported to exceed 2 %.

Conclusion

We have found multiple packaged commercial Kratom products to contain artificially elevated concentrations of 7-hydroxymitragynine, the alkaloid responsible for *M. speciosa*'s concerning mechanistic and side effect profile [20–22]. The amount of 7-hydroxymitragynine exceeded that found in naturally occurring material by up to 500 %. The recognition that 7-hydroxymitragynine is itself a metabolite further supports the notion of excessive concentrations being due to artificial addition of this psychoactive substance. Although the FDA already considers Kratom-containing supplements to be adulterated [103], federal regulations surrounding possession and use of Kratom are lacking. This study describes a unique form of product adulteration, which stresses the importance of increased dietary supplement oversight of Kratom-containing supplements.

Compliance with Ethical Standards

Conflict of Interest Authors AL, AS, CM, and BA declare that they have no conflict of interest. Authors KB and EB provide medico-legal consultation and receive royalties from UpToDate. Author EB also participates in an NIH-funded research on drugs of abuse.

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