Kansas Bureau of Investigation

Kirk D. Thompson
Director

Derek Schmidt
Attorney General

Testimony in Support of Senate Bill 282
Before the House Standing Committee on Health and Human Services
Katie Whisman, Executive Officer
Kansas Bureau of Investigation
March 8, 2018

Chairman Hawkins and Members of the Committee:

Thank you for the opportunity to testify in support of Senate Bill 282, which would update the drug schedules included in the Kansas uniform controlled substances act.

Each year, the Kansas Bureau of Investigation works very closely with the Kansas Board of Pharmacy and a multidisciplinary group of individuals to identify and address emerging drug threats in Kansas. This year, Senate Bill 282 is the product of that collaboration.

Under both the federal and state uniform controlled substances acts, there are five schedules, or lists of drugs. Schedule I drugs are those determined to have a high potential for abuse with no currently accepted medical use while Schedule V drugs are those with a low potential for abuse and a currently accepted medical use. Working closely with the Kansas Board of Pharmacy, we take great care to ensure our schedule update appropriately classifies each drug.

It is no secret that jurisdictions across the country are experiencing, firsthand, a rapidly emerging and very serious public safety threat which is commonly being referred to as "the opioid epidemic". According to the Centers for Disease Control, opioids – including prescription opioids, heroin, and fentanyl – killed more than 42,000 Americans in 2016. This surpassed any year on record. Kansas is not immune from this public health crisis.

While issues regarding prescription opioid abuse and overdose will not be addressed by SB 282, this legislation is critically important as it seeks to add several illicit and dangerous synthetic opioids to the list of controlled substances. When prescription drug abusers are unable to obtain the pharmaceuticals upon which they have become dependent, they often turn to counterfeit prescription pills or illicit drugs such as heroin. Unfortunately, these illicit drugs often contain fentanyl and/or fentanyl analogs, sometimes in deadly amounts. They pose significant risks to illicit drug users, law enforcement officers, and forensic scientists that come in contact with them.

In order to avoid an imminent hazard to the public safety, we propose adding the following synthetic opioid fentanyl compounds to Schedule I in K.S.A. 65-4105(b):

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acryl fentanyl, cyclopentyl fentanyl, cyclopropyl fentanyl, isobutyryl fentanyl, methoxyacetyl fentanyl, ocfentanil, ortho-fluorofentanyl, para-chloroisobutyryl fentanyl, para-fluorobutyryl fentanyl, para-methoxybutyryl fentanyl, tetrahydrofuranyl fentanyl, and valeryl fentanyl

We have also proposed the inclusion of MT-45, an opioid analgesic drug with pharmacological effects similar to morphine, as well as mitragynine and 7-hydroxymitragynine, which act on opioid receptors and create analgesic and euphoric effects, to Schedule I in K.S.A. 65-4105(b). An immediate precursor to fentanyl, commonly referred to as 4-ANPP, is being added to Schedule II in K.S.A. 65-4107(f).

In addition to strengthening our ability to combat fentanyl compounds and opioids, Senate Bill 282 will also help us continue our efforts to address "Designer Drugs", or those that are synthesized with the specific intent of circumventing existing drug laws by slightly altering the molecular structure of a currently controlled substance. These alterations, although often slight, produce analog substances that are equally potent in their effect on the human central nervous system. A cocktail of chemicals is often sprayed onto botanical substances and marketed as "potpourri" or "spice". When smoked, they cause users to become violently ill or lose consciousness.

Several years ago, in response to the growing trends in the use of Designer Drugs and the challenges they present in scheduling, Kansas adopted a "class" approach to drug scheduling. This year, we propose updating some existing synthetic cannabinoid class definitions to include a cyanoalkyl substitution in several classes, cyanoalkyl and an additional benzyl substitution in the indazole-3-carbaxamide class. Additionally, we propose the creation of a new indole-3-carboxamide synthetic cannabinoid class that includes a variety of potential substitutions.

There are a few "clean-up" amendments proposed to strike federal drug code numbers no longer in use and add federal drug code numbers where newly created. In accordance with Federal law, oral solutions of Dronabinol are being added to Schedule II in K.S.A. 65-4107(g). We support the Kansas Board of Pharmacy's recommendation that several anabolic steroids be added to K.S.A. 65-4109(f) in accordance with Federal law.

While SB 282 contains a number of proposed changes to the Kansas uniform controlled substances act, the conversation has recently focused solely on Kratom. To ensure the Committee has information that allows for fair and balanced contemplation of the Kratom debate, the remainder of my testimony is information important in understanding why it was proposed for inclusion in Schedule I, which includes substances for which there is no currently accepted medical use and pose a high potential for abuse.

Kratom (Mitragyna speciosa) contains over 40 different alkaloids which comprise 0.5-1.5% of the plant matter[1]. The main psychoactive alkaloid is mitragynine, which accounts for up to 66% of the plant's alkaloid content and is approximately 1/3 as potent as morphine and three times as potent as codeine[1]. While less abundant, 7-hydroxymitragynine accounts for roughly 2% of the

[1] Lydecker, Sharma, et.al., "Suspected Adulteration of Commercial Kratom Products with 7-Hydroxymitragynine, J Med Toxicol., 2016 Dec, 12(4): 341-349

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plant's alkaloid content; it has been characterized as a "highly potent and addictive plant alkaloid"[1] with a potency 4.4 to 5.7 times more than that of morphine[2].

Mitragynine and 7-hydroxymitragynine are opioid receptor agonists that produce opioid-like analgesic, euphoric, and sedating effects. Both are considered opioids because they bind with one or more of the opioid receptors in the body and cause an effect. They are not structurally related to compounds that come from opium such as morphine, or derived from opium compounds so they are not opiates. To put this in context, fentanyl is an opioid but not an opiate.

Acute and chronic mitragynine use has demonstrated addiction-related and cognitive-impairing effects.[3] One study concluded mitragynine "has a significant abuse and addiction potential and can cause profound emotional and cognitive impairments which resemble that of opiate and psychostimulant drugs." This finding suggests its classification as a harmful drug is appropraite.[3]

Despite what you heard earlier in the week, Kratom use has been linked to dependence and withdrawal symptoms. A cross sectional survey of 293 regular Kratom users in Malaysia showed that more than half of regular users developed severe Kratom dependence problems, while 45% showed a moderate Kratom dependence. Physical withdrawal symptoms commonly experienced include muscle spasms and pain, sleeping difficulty, watery eyes/nose, hot flashes, fever, decreased appetite, and diarrhoea. Psychological withdrawal symptoms commonly reported were restlessness, tension, anger, sadness, and nervousness.[4]

Lending to the abuse liability and addictive potential of Kratom products[4], there are strong indications that commercial Kratom products have been adulterated by artificially increasing the concentration of 7-hydroxymitragynine. In one study, 7/8 encapsulated Kratom supplements revealed the 7-hydroxymitragynine concentration to be 109-520% more concentrated than in the naturally occurring Kratom leaf[1].

Under current Kansas law, it is legal for mitragynine and 7-hydroxymitragynine to be sold in any form. There is nothing to stop one from extracting the psychoactive substances from the plant and making the pure form available for ingestion. This isn't a speculative scenario. Last month the Food and Drug Administration (FDA) moved to prevent the sale of a product called "Mitrasafe," which is advertised as an extract of 99% pure mitragynine[5]. Although its direct sale with the United States is currently stalled, Kratom products are readily available for purchase on the internet.

Kratom is viewed as a "legal alternative" to opioid use and, as you heard Monday, is also used to help treat addiction in the absence of medical supervision. There is no reliable evidence to

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[2] Masumoto, Hatori, et. al. "Involvement of u-opioid receptors in the antinociception and inhibition of gastrointestinal transit induced by 7-hydroxymitragynine, isolated from Thai herbal medicine Mitragyna speciosa." European Journal of Pharmacology, Volume 549, November 2006, 63-70

[3] Yusoff, Nurul, et.al "Abuse potential and adverse cognitive effects of mitragynine (kratom)", Addiction Biology (2014) 21:98-110

[4] Singh, Muller, et.al, "Kratom (Mitrayna speciosa) dependence, withdrawal symptoms and craving in regular users", Drug and Alcohol Dependence 139 (2014) 132-137

[5] Kroll, "FDA Warns Against Launch Of The Kratom Extract Mitrasafe, Citing Misleading Regulatory And Drug Claims, Forbes / Pharma & Healthcare / #Medicine https://www.forbes.com/sites/davidkroll/2018/02/27/fda-warns-against-launch-of-kratom-extract-mitrasafe-cites-misleading-regulatory-and-drug-claims/#4e422c482d5a Accessed March 7, 2018

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support Kratom's use as a treatment for opioid abuse disorder. The interactions of Kratom with other drugs is largely unstudied and there is no research on what effects long term Kratom use has on brain chemistry or how it may alter the structure of the receptors. Medical applications should be accepted only after research and approval by the FDA.

It is for the many reasons articulated above that the KBI and Kansas Board of Pharmacy have recommended the inclusion of both mitragynine and 7-hydroxymitragynine in Schedule I.

While it may be of little influence in your contemplation of this important public policy decision, mitragynine, 7-hydroxymitragynine and/or Kratom are under some form of control in Denmark, Lithuania, Latvia, Romania, Poland, Sweden, Australia, Malaysia, Burma, and Thiland, New Zeland, South Korea, Germany and Israel. It is of note that these are countries over which the FDA has no control.

On Monday, this Committee heard the Legislative Director of the American Kratom Association acknowledge that in light of an import ban on Kratom, which was enacted in 2012, products brought into and sold within the United States are most likely imported under false pretenses, being marked ***"Not intended for human consumption."*** Attached to my testimony you will find photographs of products located in the residence of a northeast Kansas accidental overdose victim on March 4, 2018. Despite the packaging, these products are clearly being sold and intended for human consumption. This labeling is not a direction or warning to users but a tactic to avoid detection and enforcement. We saw similar tactics with K-2, potpourri, and in 2016 the deadly U-47700. Each of these products was recognized for what they were – illicit drugs. In an effort to protect the health and safety of our citizens, they were controlled accordingly.

While Kratom is not currently controlled in the United States, it is listed on the Drug Enforcement Agency's list of Drugs and Chemicals of Concern. According to the American Kratom Association's website, Kratom is illegal in Wisconsin, Indiana Tennessee, Arkansas, Alabama, Vermont, Rhode Island and Washington DC.

I urge you to objectively weigh both sides of this discussion as you contemplate whether the State of Kansas should add its name to the list of states that have made public policy decisions to protect the health and welfare of its citizens by controlling the active ingredients in Kratom.

In closing, I'd like to emphasize that timely passage of SB 282 is important to our state's ability to manage the threats dangerous substances pose to the health and safety of our citizens. For that reason, we kindly ask that the contents of SB 282 not be confused with or married to other public policy matters.

Thank you for your time. I would be happy to stand for questions.

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Suspected Adulteration of Commercial Kratom Products with 7-Hydroxymitragynine

Alicia G. Lydecker,[1] Abhisheak Sharma,[2] Christopher R. McCurdy,[3] Bonnie A. Avery,[2,3] Kavita M. Babu,[1] and Edward W. Boyer[1]

[1]Division of Medical Toxicology, Department of Emergency Medicine, University of Massachusetts Medical School, 55 Lake Ave North, Worcester, MA 01655 USA
[2]Department of Pharmaceutics and Drug Delivery, School of Pharmacy, University of Mississippi, 104A Faser Hall, P.O. Box 1848, University, MS 38677-1848 USA
[3]Department of BioMolecular Sciences, School of Pharmacy, University of Mississippi, 417 Faser Hall, P.O. Box 1848, University, MS 38677-1848 USA
Alicia G. Lydecker, Phone: 845-728-5382, Email: alicia.lydecker@gmail.com.
Corresponding author.

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Abstract

Go to:

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

Background

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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].

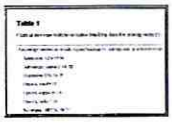

Table 1

Central nervous system receptor binding data for mitragynine [10]

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.

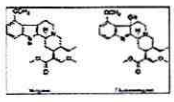

Fig. 1

Structures of mitragynine and 7-hydroxymitragynine

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.

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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™ (Miami, FL, USA) red, Phoria™ green, Phoria™ regular, Phoria™ Borneo white vein, Phoria™ Borneo red vein, Phoria™ Borneo green vein, Phoria™ maeng da blue lotus, Phoria™ 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

Go to:

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.

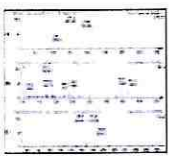

Fig. 2
Product ion spectra of **a** mitragynine (*m/z* 399.39), **b** 7-hydroxymitragynine (415.34), and **c** internal standard (*m/z* 294.37)

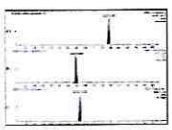

Fig. 3
Representative MRM chromatogram of **a** mitragynine (1.0 ng/mL; LLOQ), **b** 7-hydroxymitragynine (1.0 ng/mL; LLOQ), and **c** IS (10 ng/mL)

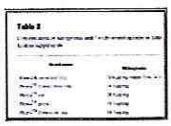

Table 2
Concentration of mitragynine and 7-hydroxymitragynine in naturally occurring Kratom leaf and marketed Kratom supplements

Discussion

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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 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 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

Go to:

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

Go to:

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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References

Go to:

1. Suwanlert S. A study of Kratom eaters in Thailand. Bull Narc. 1975;27(3):21–7. [PubMed]

2. Warner ML, Kaufman NC, Grundmann O. The pharmacology and toxicology of Kratom: from traditional herb to drug of abuse. Int J Legal Med. 2016;130(1):127–38. [PubMed]

3. Jansen K, Prast CJ. Ethnopharmacology of Kratom and the Mitragyna alkaloids. J Ethnopharmacol. 1988;23(1):115–119. [PubMed]

4. Grewal KS. The effect of mitragynine on man. Br J Med Psychol. 2011;12(1):41–58. doi: 10.1111/j.2044-8341.1932.tb01062.x. [Cross Ref]

5. Matsumoto K, Mizowaki M, Suchitra T, Takayama H, Sakai S-I, Aimi N, et al. Antinociceptive action of mitragynine in mice: evidence for the involvement of supraspinal opioid receptors. Life Sci. 1996;59(14):1149–1155. doi: 10.1016/0024-3205(96)00432-8. [PubMed] [Cross Ref]

6. Kratom (Mitragyna speciosa) Drug Profile. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA). 2015. http://www.emcdda.europa.eu/publications/drug-profiles/kratom. Accessed 16 Jan 2016.

7. Kratom (Mitragyna speciosa Korth). Drug Enforcement Administration, Office of Diversion Control, Drug & Chemical Evaluation Section. 2013. http://www.deadiversion.usdoj.gov/drug_chem_info/kratom.pdf. Accessed 16 Jan 2016.

8. Special Report: Opiates and Related Drugs Reported in NFLIS, 2009–2014. U.S. Department Of Justice, Drug Enforcement Administration, Office of Diversion Control. 2016. https://www.nflis.deadiversion.usdoj.gov/DesktopModules/ReportDownloads/Reports/NFLIS-SR-Opioids-Rev.pdf. Accessed 21 June 2016.

9. Huus K. Asian leaf "kratom" making presence felt in US emergency rooms. NBC News. 2012. http://usnews.nbcnews.com/_news/2012/03/19/10760892-asian-leaf-kratom-making-presence-felt-in-us-emergency-rooms. Accessed 10 May 2016.

10. Boyer EW, Babu KM, Adkins JE, McCurdy CR, Halpern JH. Self-treatment of opioid withdrawal using Kratom (Mitragyna speciosa korth) Addiction. 2008;103(6):1048–1050. doi: 10.1111/j.1360-0443.2008.02209.x. [PMC free article] [PubMed] [Cross Ref]

11. Prozialeck WC, Jivan JK, Andurkar SV. Pharmacology of Kratom: an emerging botanical agent with stimulant, analgesic and opioid-like effects. J Am Osteopath Assoc. 2012;112(12):792–799. [PubMed]

12. Dargan P, Wood D. Novel Psychoactive Substances. Boston: Academic; 2013.

13. Hassan Z, Muzaimi M, Navaratnam V, Yusoff NHM, Suhaimi FW, Vadivelu R, et al. From Kratom to mitragynine and its derivatives: physiological and behavioural effects related to use, abuse, and addiction. Neurosci Biobehav Rev. 2013;37(2):138–151. doi: 10.1016/j.neubiorev.2012.11.012. [PubMed] [Cross Ref]

14. Cinosi E, Martinotti G, Simonato P, Singh D, Demetrovics Z, Roman-Urrestarazu A, et al. Following "the roots" of Kratom (Mitragyna speciosa): the evolution of an enhancer from a traditional use to increase work and productivity in Southeast Asia to a recreational psychoactive drug in western countries. Biomed Res Int. 2015;2015:1–11. [PMC free article] [PubMed]

15. Shellard EJ. The alkaloids of Mitragyna with special reference to those of Mitragyna speciosa, Korth. Bull Narc. 1974;26(2):41–55. [PubMed]

16. Ponglux D, Wongseripipatana S, Takayama H, Kikuchi M, Kurihara M, Kitajima M, et al. A new indole alkaloid, 7 alpha-hydroxy-7H-mitragynine, from Mitragyna speciosa in Thailand. Planta Med. 1994;60(06):580–581. doi: 10.1055/s-2006-959578. [PubMed] [Cross Ref]

17. Thongpradichote S, Matsumoto K, Tohda M, Takayama H, Aimi N, Sakai S, et al. Identification of opioid receptor subtypes in antinociceptive actions of supraspinally-administered mitragynine in mice. Life Sci. 1998;62(16):1371–1378. doi: 10.1016/S0024-3205(98)00075-7. [PubMed] [Cross Ref]

18. Matsumoto K, Mizowaki M, Suchitra T, Murakami Y, Takayama H, Sakai S-I, et al. Central antinociceptive effects of mitragynine in mice: contribution of descending noradrenergic and serotonergic systems. Eur J Pharmacol. 1996;317(1):75–81. doi: 10.1016/S0014-2999(96)00714-5. [PubMed] [Cross Ref]

19. Gregory TB. Hydromorphone: evolving to meet the challenges of today's health care environment. Clin Ther. 2013;35(12):2007–2027. doi: 10.1016/j.clinthera.2013.09.027. [PubMed] [Cross Ref]

20. Matsumoto K, Horie S, Ishikawa H, Takayama H, Aimi N, Ponglux D, et al. Antinociceptive effect of 7-hydroxymitragynine in mice: discovery of an orally active opioid analgesic from the Thai medicinal herb Mitragyna speciosa. Life Sci. 2004;74(17):2143–2155. doi: 10.1016/j.lfs.2003.09.054. [PubMed] [Cross Ref]

21. Takayama H, Ishikawa H, Kurihara M, Kitajima M, Aimi N, Ponglux D, et al. Studies on the synthesis and opioid agonistic activities of mitragynine-related indole alkaloids: discovery of opioid agonists structurally different from other opioid ligands. J Med Chem. 2002;45(9):1949–1956. doi: 10.1021/jm010576e. [PubMed] [Cross Ref]

22. Horie S, Koyama F, Takayama H, Ishikawa H, Aimi N, Ponglux D, et al. Indole alkaloids of a Thai medicinal herb, Mitragyna speciosa, that has opioid agonistic effect in guinea-pig ileum. Planta Med. 2005;71(3):231–236. doi: 10.1055/s-2005-837822. [PubMed] [Cross Ref]

23. Adkins JE, Boyer EW, McCurdy CR. Mitragyna speciosa, a psychoactive tree from Southeast Asia with opioid activity. Curr Top Med Chem. 2011;11(9):1165–1175. doi: 10.2174/156802611795371305. [PubMed] [Cross Ref]

24. Roche KM, Hart K, Sangalli B. Kratom: a case of a legal high. Clin Toxicol. 2008;46(7):598.

25. McWhirter L, Morris S. A case report of inpatient detoxification after Kratom (Mitragyna speciosa) dependence. Eur Addict Res. 2010;16(4):229–231. doi: 10.1159/000320288. [PubMed] [Cross Ref]

26. Babu KM, McCurdy CR, Boyer EW. Opioid receptors and legal highs: *Salvia divinorum* and Kratom. Clin Toxicol (Phila) 2008;46(2):146–152. doi: 10.1080/15563650701241795. [PubMed] [Cross Ref]

27. Matsumoto K, Horie S, Takayama H, Ishikawa H, Aimi N, Ponglux D, et al. Antinociception, tolerance and withdrawal symptoms induced by 7-hydroxymitragynine, an alkaloid from the Thai medicinal herb Mitragyna speciosa. Life Sci. 2005;78(1):2–7. doi: 10.1016/j.lfs.2004.10.086. [PubMed] [Cross Ref]

28. Narita M, Funada M, Suzuki T. Regulations of opioid dependence by opioid receptor types. Pharmacol Ther. 2001;89(1):1–15. doi: 10.1016/S0163-7258(00)00099-1. [PubMed] [Cross Ref]

29. Matthes HW, Maldonado R, Simonin F, Valverde O, Slowe S, Kitchen I, et al. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature. 1996;383(6603):819–823. doi: 10.1038/383819a0. [PubMed] [Cross Ref]

30. Kikura-Hanajiri R, Kawamura M, Maruyama T, Kitajima M, Takayama H, Goda Y. Simultaneous analysis of mitragynine, 7-hydroxymitragynine, and other alkaloids in the psychotropic plant "Kratom" (Mitragyna speciosa) by LC-ESI-MS. Forensic Toxicol. 2009;27(2):67–74. doi: 10.1007/s11419-009-0070-5. [Cross Ref]

31. Grewal KS. Observations on the pharmacology of mitragynine. American Society for Pharmacology and Experimental Therapeutics. J Pharmacol Exp Ther. 1932;46(3):251–271.

32. Goh TB, Koh RY, Mordi MN, Mansor SM. Antioxidant value and antiproliferative efficacy of mitragynine and a silane reduced analogue. Asian Pac J Cancer Prev. 2014;15(14):5659–5665. doi: 10.7314/APJCP.2014.15.14.5659. [PubMed] [Cross Ref]

33. Parthasarathy S, Bin Azizi J, Ramanathan S, Ismail S, Sasidharan S, Said MI, et al. Evaluation of antioxidant and antibacterial activities of aqueous, methanolic and alkaloid extracts from Mitragyna speciosa (Rubiaceae Family) leaves. Molecules. Mol Divers Preserv Int. 2009;14(10):3964–3974. [PubMed]

34. Utar Z, Majid MIA, Adenan MI, Jamil MFA, Lan TM. Mitragynine inhibits the COX-2 mRNA expression and prostaglandin E_2 production induced by lipopolysaccharide in RAW264.7 macrophage cells. J Ethnopharmacol. 2011;136(1):75–82. doi: 10.1016/j.jep.2011.04.011. [PubMed] [Cross Ref]

35. Burkill IH, Haniff M. Malay village medicine, prescriptions collected. Gard Bull. 1930;6:165–321.

36. Tanguay P. Kratom in Thailand. Legis Reform Drug Policies. 2011;13:1–16.

37. Idayu NF, Hidayat MT, Moklas MAM, Sharida F, Raudzah ARN, Shamima AR, et al. Antidepressant-like effect of mitragynine isolated from Mitragyna speciosa Korth in mice model of depression. Phytomedicine. 2011;18(5):402–407. doi: 10.1016/j.phymed.2010.08.011. [PubMed] [Cross Ref]

38. Harizal SN, Mansor SM, Hasnan J, Tharakan JKJ, Abdullah J. Acute toxicity study of the standardized methanolic extract of Mitragyna speciosa Korth in rodent. J Ethnopharmacol. 2010;131 (2):404–409. doi: 10.1016/j.jep.2010.07.013. [PubMed] [Cross Ref]

39. Kumarnsit E, Keawpradub N, Nuankaew W. Effect of Mitragyna speciosa aqueous extract on ethanol withdrawal symptoms in mice. Fitoterapia. 2007;78(3):182–185. doi: 10.1016/j.fitote.2006.11.012. [PubMed] [Cross Ref]

40. Vicknasingam B, Narayanan S, Beng GT, Mansor SM. The informal use of ketum (Mitragyna speciosa) for opioid withdrawal in the northern states of peninsular Malaysia and implications for drug substitution therapy. Int J Drug Policy. 2010;21(4):283–288. doi: 10.1016/j.drugpo.2009.12.003. [PubMed] [Cross Ref]

41. Ahmad K, Aziz Z. Mitragyna speciosa use in the northern states of Malaysia: a cross-sectional study. J Ethnopharmacol. 2012;141(1):446–450. doi: 10.1016/j.jep.2012.03.009. [PubMed] [Cross Ref]

42. Shellard EJ. Ethnopharmacology of Kratom and the Mitragyna alkaloids. J Ethnopharmacol. 1989;25(1):123–124. doi: 10.1016/0378-8741(89)90053-6. [PubMed] [Cross Ref]

43. Erowid. Kratom Dosage. The Vaults of Erowid. 2015. https://www.erowid.org/plants/kratom/kratom_dose.shtml. Accessed 23 Jan 2016.

44. Erowid. Kratom (Mitragyna speciosa) Effects. The Vaults of Erowid. 2016. https://www.erowid.org/plants/kratom/kratom_effects.shtml. Accessed 23 Jan 2016.

45. Thuan LC. Addiction to Mitragyna speciosa. Proc Alumni Assoc. 1957;10:322–324.

46. Yusoff NHM, Suhaimi FW, Vadivelu RK, Hassan Z, Rümler A, Rotter A, et al. Abuse potential and adverse cognitive effects of mitragynine (Kratom. Addict Biol. 2014;21:98–110. doi: 10.1111/adb.12185. [PubMed] [Cross Ref]

47. Nelsen JL, Lapoint J, Hodgman MJ, Aldous KM. Seizure and coma following Kratom (Mitragynina speciosa Korth) exposure. J Med Toxicol. 2010;6(4):424–426. doi: 10.1007/s13181-010-0079-5. [PMC free article] [PubMed] [Cross Ref]

48. Lu J, Wei H, Wu J, Jamil MFA, Tan ML, Adenan MI, et al. Evaluation of the cardiotoxicity of mitragynine and its analogues using human induced pluripotent stem cell-derived cardiomyocytes. Clin Toxicol. 2014;9(12):e115648. [PMC free article] [PubMed]

49. Cabellon M, Aris R, Pathak V. Adult respiratory distress syndrome secondary to the use of herbal drug kratom. Am J Respir Crit Care Med. 2014;189:A6492.

50. Sheleg SV, Collins GB. A coincidence of addiction to "Kratom" and severe primary hypothyroidism. J Addict Med. 2011;5(4):300–301. doi: 10.1097/ADM.0b013e318221fbfa. [PubMed] [Cross Ref]

51. Kapp FG, Maurer HH, Auwärter V, Winkelmann M, Hermanns-Clausen M. Intrahepatic cholestasis following abuse of powdered Kratom (Mitragyna speciosa) J Med Toxicol. 2011;7(3):227–231. doi: 10.1007/s13181-011-0155-5. [PMC free article] [PubMed] [Cross Ref]

52. Lu J, Wei H, Wu J, Jamil M, Tan ML, Adenan MI. Evaluation of the cardiotoxicity of mitragynine and its analogues using human induced pluripotent stem cell-derived cardiomyocytes. PLoS One. 2014;9(12):e115648. doi: 10.1371/journal.pone.0115648. [PMC free article] [PubMed] [Cross Ref]

53. Holler JM, Vorce SP, McDonough-Bender PC, Joseph Magluilo J, Solomon CJ, Levine B. A drug toxicity death involving propylhexedrine and mitragynine. J Anal Toxicol. 2011;35(1):54–59. doi: 10.1093/anatox/35.1.54. [PubMed] [Cross Ref]

54. Tungtananuwat W, Lawanprasert S. Fatal 4×100: homemade Kratom juice cocktail. J Health Res. 2010;24(1):43–47.

55. Neerman MF, Frost RE, Deking J. A drug fatality involving Kratom. J Forensic Sci. 2013;58 (s1):S278–S279. doi: 10.1111/1556-4029.12009. [PubMed] [Cross Ref]

56. McIntyre IM, Trochta A, Stolberg S, Campman SC. Mitragynine "Kratom" related fatality: a case report with postmortem concentrations. J Anal Toxicol. 2015;39(2):152–155. doi: 10.1093/jat/bku137. [PubMed] [Cross Ref]

57. Kronstrand R, Roman M, Thelander G, Eriksson A. Unintentional fatal intoxications with mitragynine and O-desmethyltramadol from the herbal blend krypton. J Anal Toxicol. 2011;35(4):242–247. doi: 10.1093/anatox/35.4.242. [PubMed] [Cross Ref]

58. Scott TM, Yeakel JK, Logan BK. Identification of mitragynine and O-desmethyltramadol in Kratom and legal high products sold online. Drug Test Anal. 2014;6(9):959–963. doi: 10.1002/dta.1673. [PubMed] [Cross Ref]

59. Dietary Supplement Health and Education Act of 1994. National Institutes of Health Office of Dietary Supplements. 1994. https://ods.od.nih.gov/About/DSHEA_Wording.aspx. Accessed 31 Jan 2016.

60. Petróczi A, Ocampo JAV, Shah I, Jenkinson C, New R, James RA, et al. Russian roulette with unlicensed fat-burner drug 2,4-dinitrophenol (DNP): evidence from a multidisciplinary study of the internet, bodybuilding supplements and DNP users. Subst Abuse Treat Prev Policy. 2015;10(1). [PMC free article] [PubMed]

61. Rebiere H, Guinot P, Civade C, Bonnet P-A, Nicolas A. Detection of hazardous weight-loss substances in adulterated slimming formulations using ultra-high-pressure liquid chromatography with diode-array detection. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2012;29(2):161–171. doi: 10.1080/19440049.2011.638676. [PubMed] [Cross Ref]

62. Venhuis BJ, Zwaagstra ME, Van den Berg J. Trends in drug substances detected in illegal weight-loss medicines and dietary supplements. RIVM Report. 2009;370030002:1–37.

63. Jung J, Hermanns-Clausen M, Weinmann W. Anorectic sibutramine detected in a Chinese herbal drug for weight loss. Forensic Sci Int. 2006;161(2–3):221–222. doi: 10.1016/j.forsciint.2006.02.052. [PubMed] [Cross Ref]

64. Wang J, Chen B, Yao S. Analysis of six synthetic adulterants in herbal weight-reducing dietary supplements by LC electrospray ionization-MS. Food Addit Contam. 2008;25(7):822–830. doi: 10.1080/02652030801946553. [PubMed] [Cross Ref]

65. Vaysse J, Balayssac S, Gilard V, Desoubdzanne D, Malet-Martino M, Martino R. Analysis of adulterated herbal medicines and dietary supplements marketed for weight loss by DOSY 1H-NMR. Food Addit Contam. 2010;27(7):903–916. doi: 10.1080/19440041003705821. [PubMed] [Cross Ref]

66. Shi Y, Sun C, Gao B, Sun A. Development of a liquid chromatography tandem mass spectrometry method for simultaneous determination of eight adulterants in slimming functional foods. J Chromatogr A. 2011;1218(42):7655–7662. doi: 10.1016/j.chroma.2011.08.038. [PubMed] [Cross Ref]

67. De Carvalho LM, Cohen PA, Silva CV, Moreira APL, Falcão TM, Molin TRD, et al. A new approach to determining pharmacologic adulteration of herbal weight loss products. Food Addit Contam: Part A. 2012;29(11):1661–1667. doi: 10.1080/19440049.2012.706834. [PubMed] [Cross Ref]

68. Park S-R, Lee JG, Roh SH, Kim G, Kwon CH, Park HR, et al. Determination of PDE-5 inhibitors and appetite suppressants in adulterated dietary supplements using LC/PDA and LC/MS. Food Addit Contam: Part B. 2012;5(1):29–32. doi: 10.1080/19393210.2012.656706. [PubMed] [Cross Ref]

69. Song F, Monroe D, El-Demerdash A, Palmer C. Screening for multiple weight loss and related drugs in dietary supplement materials by flow injection tandem mass spectrometry and their confirmation by liquid chromatography tandem mass spectrometry. J Pharm Biomed Anal. 2014;88:136–143. doi: 10.1016/j.jpba.2013.08.031. [PubMed] [Cross Ref]

70. Kim HJ, Lee JH, Park HJ, Cho S-H, Cho S, Kim WS. Monitoring of 29 weight loss compounds in foods and dietary supplements by LC-MS/MS. Food Addit Contam: Part A. 2014;31(5):777–783. doi: 10.1080/19440049.2014.888497. [PubMed] [Cross Ref]

71. Hachem R, Malet-Martino M, Gilard V. First identification and quantification of lorcaserin in an herbal slimming dietary supplement. J Pharm Biomed Anal. 2014;98:94–99. doi: 10.1016/j.jpba.2014.05.003. [PubMed] [Cross Ref]

72. Reeuwijk NM, Venhuis BJ, de Kaste D, Hoogenboom RLAP, Rietjens IMCM, Martena MJ. Active pharmaceutical ingredients detected in herbal food supplements for weight loss sampled on the Dutch market. Food Addit Contam: Part A. 2014;31(11):1783–1793. doi: 10.1080/19440049.2014.958574. [PubMed] [Cross Ref]

73. Mathon C, Ankli A, Reich E, Bieri S, Christen P. Screening and determination of sibutramine in adulterated herbal slimming supplements by HPTLC-UV densitometry. Food Addit Contam: Part A. 2013;31(1):15–20. doi: 10.1080/19440049.2013.861934. [PubMed] [Cross Ref]

74. Strano-Rossi S, Odoardi S, Castrignanò E, Serpelloni G, Chiarotti M. Liquid chromatography–high resolution mass spectrometry (LC–HRMS) determination of stimulants, anorectic drugs and phosphodiesterase 5 inhibitors (PDE5I) in food supplements. J Pharm Biomed Anal. 2015;106:144–152. doi: 10.1016/j.jpba.2014.06.011. [PubMed] [Cross Ref]

75. Maughan RJ. Contamination of dietary supplements and positive drug tests in sport. J Sports Sci. 2005;23(9):883–889. doi: 10.1080/02640410400023258. [PubMed] [Cross Ref]

76. Maughan RJ, Greenhaff PL, Hespel P. Dietary supplements for athletes: emerging trends and recurring themes. J Sports Sci. 2011;29(suppl 1):S57–S66. doi: 10.1080/02640414.2011.587446. [PubMed] [Cross Ref]

77. Geyer H, Parr MK, Mareck U, Reinhart U, Schrader Y, Schänzer W. Analysis of non-hormonal nutritional supplements for anabolic-androgenic steroids—results of an international study. Int J Sports Med. 2004;25(2):124–129. doi: 10.1055/s-2004-819955. [PubMed] [Cross Ref]

78. Geyer H, Parr MK, Koehler K, Mareck U, Schänzer W, Thevis M. Nutritional supplements cross-contaminated and faked with doping substances. J Mass Spectrom. 2008;43(7):892–902. doi: 10.1002/jms.1452. [PubMed] [Cross Ref]

79. van der Merwe PJ, Grobbelaar E. Unintentional doping through the use of contaminated nutritional supplements. S Afr Med J. 2005;95(7):510–511. [PubMed]

80. Pipe A, Ayotte C. Nutritional supplements and doping. Clin J Sport Med. 2002;12(4):245–249. doi: 10.1097/00042752-200207000-00008. [PubMed] [Cross Ref]

81. Campbell N, Clark JP, Stecher VJ, Thomas JW, Callanan AC, Donnelly BF, et al. Adulteration of purported herbal and natural sexual performance enhancement dietary supplements with synthetic phosphodiesterase type 5 inhibitors. J Sex Med. 2013;10(7):1842–1849. doi: 10.1111/jsm.12172. [PubMed] [Cross Ref]

82. Gratz SR, Flurer CL, Wolnik KA. Analysis of undeclared synthetic phosphodiesterase-5 inhibitors in dietary supplements and herbal matrices by LC–ESI–MS and LC–UV. J Pharm Biomed Anal. 2004;36(3):525–533. doi: 10.1016/j.jpba.2004.07.004. [PubMed] [Cross Ref]

83. Ng CS, Law TY, Cheung YK, Ng PC, Choi KK. Development of a screening method for the detection of analogues of sildenafil and vardenafil by the use of liquid chromatograph coupled with triple quadrupole linear ion trap mass spectrometer. analytical methods. Royal Soc Chem. 2010;2(7):890–896.

84. Savaliya AA, Shah RP, Prasad B, Singh S. Screening of Indian aphrodisiac ayurvedic/herbal healthcare products for adulteration with sildenafil, tadalafil and/or vardenafil using LC/PDA and extracted ion LC–MS/TOF. J Pharm Biomed Anal. 2010;52(3):406–409. doi: 10.1016/j.jpba.2009.05.021. [PubMed] [Cross Ref]

85. Zhang Y, Huang Z, Ding L, Yan H, Wang M, Zhu S. Simultaneous determination of yohimbine, sildenafil, vardenafil and tadalafil in dietary supplements using high-performance liquid chromatography-tandem mass spectrometry. J Sep Sci. 2010;33(14):2109–14. doi: 10.1002/jssc.200900841. [PubMed] [Cross Ref]

86. Toomey VM, Litzau JJ, Flurer CL. Isolation and structural characterization of two tadalafil analogs found in dietary supplements. J Pharm Biomed Anal. 2012;59:50–57. doi: 10.1016/j.jpba.2011.09.038. [PubMed] [Cross Ref]

87. Ren Y, Wu C, Zhang J. Simultaneous screening and determination of 18 illegal adulterants in herbal medicines and health foods for male sexual potency by ultra-fast liquid chromatography-electrospray ionization tandem mass spectrometry. J Sep Sci. 2012;35(21):2847–2857. doi: 10.1002/jssc.201200280. [PubMed] [Cross Ref]

88. Vaysse J, Gilard V, Balayssac S, Zedde C, Martino R, Malet-Martino M. Identification of a novel sildenafil analogue in an adulterated herbal supplement. J Pharm Biomed Anal. 2012;59:58–66. doi: 10.1016/j.jpba.2011.10.001. [PubMed] [Cross Ref]

89. Jankovics P, Lohner S, Darcsi A, Németh-Palotás J, Béni S. Detection and structure elucidation of hydroxythiovardenafil as an adulterant in a herbal dietary supplement. J Pharm Biomed Anal. 2013;74:83–91. doi: 10.1016/j.jpba.2012.10.013. [PubMed] [Cross Ref]

90. Lee E-S, Kim JW, Lee JH, Han KM, Cho S, Hwang I, et al. Identification of a new tadalafil analogue found in a dietary supplement. Food Addit Contam: Part A. 2013;30(4):621–626. doi: 10.1080/19440049.2013.766766. [PubMed] [Cross Ref]

91. Lee E-S, Lee JH, Han KM, Kim JW, Hwang IS, Cho S, et al. Simultaneous determination of 38 phosphodiesterase-5 inhibitors in illicit erectile dysfunction products by liquid chromatography –electrospray ionization-tandem mass spectrometry. J Pharm Biomed Anal. 2013;83:171–178. doi: 10.1016/j.jpba.2013.05.009. [PubMed] [Cross Ref]

92. Lebel P, Gagnon J, Furtos A, Waldron KC. A rapid, quantitative liquid chromatography-mass spectrometry screening method for 71 active and 11 natural erectile dysfunction ingredients present in potentially adulterated or counterfeit products. J Chromatogr A. 2014;1343:143–151. doi: 10.1016/j.chroma.2014.03.078. [PubMed] [Cross Ref]

93. Damiano F, Silva C, Gregori A, Vacondio F, Mor M, Menozzi M, et al. Analysis of illicit dietary supplements sold in the Italian market: identification of a sildenafil thioderivative as adulterant using UPLC–TOF/MS and GC/MS. Sci Justice. 2014;54(3):228–237. doi: 10.1016/j.scijus.2014.02.009. [PubMed] [Cross Ref]

94. Thailand Narcotics Control Division. Table of controlled narcotic drugs in Thailand. 2015. http://narcotic.fda.moph.go.th/welcome/wp-content/uploads/2015/10/NARCO-list-update-05.10.2015.pdf. Accessed 10 May 2016.

95. Adulyadej B. Narcotics Act. Thailand Narcotics Control Division. 1979. http://narcotic.fda.moph.go.th/english/wp-content/uploads/2012/11/Narcotics-Act-B.E.2522.pdf. Accessed 10 May 2016.

96. House Enrolled Act No. 1196. In: Second Regular Session 117th General Assembly. 2012. http://www.in.gov/legislative/bills/2012/HE/HE1196.1.html. Accessed 10 May 2016.

97. Beavers M. Senate Bill 48. General Assembly of the State of Tennessee. 2013. http://www.capitol.tn.gov/Bills/108/Bill/SB0048.pdf. Accessed 10 May 2016.

98. Vermont Department of Health. Regulated Drug Rule. 2016. http://healthvermont.gov/regs/documents/regulated_drugs_rule.pdf. Accessed 10 May 2016.

99. Louisiana State Legislature. RS 40:989.3. 2012. https://legis.la.gov/legis/Law.aspx?d=814206. Accessed 10 May 2016.

100. Uniform Controlled Substances Act. Wisconsin State Legislature. 2016. http://docs.legis.wisconsin.gov/statutes/statutes/961/II/14. Accessed 10 May 2016.

101. House File 2355 [Internet]. [cited 2016 May 10]. Available from: http://coolice.legis.iowa.gov/linc/85/external/HF2355_Introduced.html.

102. Kratom Control Act. Illinois General Assembly. 2015. http://www.ilga.gov/legislation/ilcs/ilcs3.asp?ActID=3573&ChapterID=53. Accessed 10 May 2016.

103. U.S. Food and Drug Administration. Import Alert 54–15. 2016. http://www.accessdata.fda.gov/cms_ia/importalert_1137.html. Accessed 10 May 2016.

104. U.S. Food and Drug Administration. New Dietary Ingredients Notification Process. 2016. http://www.fda.gov/Food/DietarySupplements/NewDietaryIngredientsNotificationProcess/default.htm. Accessed 10 May 2016.

105. United States Government Publishing Office. 21- Food and Drugs §§ 342. https://www.gpo.gov/fdsys/pkg/USCODE-2010-title21/pdf/USCODE-2010-title21-chap9-subchapIV-sec342.pdf. Accessed 19 May 2016.

106. U.S. Food and Drug Administration. US Marshals Seize Dietary Supplements Containing Kratom. 2016. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm480344.htm. Accessed 10 May 2016.

107. U.S. Department of Justice Drug Enforcement Administration. Drugs of Abuse. 2015. http://www.dea.gov/pr/multimedia-library/publications/drug_of_abuse.pdf#page=84. Accessed 10 May 2016.

108. Yoburn BC, Shah S, Chan K, Duttaroy A, Davis T. Supersensitivity to opioid analgesics following chronic opioid antagonist treatment: relationship to receptor selectivity. Pharmacol Biochem Behav. 1995;51(2–3):535–539. doi: 10.1016/0091-3057(94)00375-S. [PubMed] [Cross Ref]

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PRECLINICAL STUDY

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Abuse potential and adverse cognitive effects of mitragynine (kratom)

Nurul H. M. Yusoff[1]*, Farah W. Suhaimi[1]*, Raja K. Vadivelu[1,2]*, Zurina Hassan[1]*, Anne Rümler[3]*, Andrea Rotter[3], Davide Amato[3], Hans C. Dringenberg[4], Sharif M. Mansor[1], Visweswaran Navaratnam[1]* & Christian P. Müller[3]*

Centre for Drug Research, Universiti Sains Malaysia, Malaysia[1], Eskitis Institute for Drug Discovery, Griffith University, Australia[2], Department of Psychiatry and Psychotherapy, University Clinic, Friedrich-Alexander-University Erlangen-Nuremberg, Germany[3] and Department of Psychology and Centre for Neuroscience Studies, Queen's University, Canada[4]

ABSTRACT

Mitragynine is the major psychoactive alkaloid of the plant kratom/ketum. Kratom is widely used in Southeast Asia as a recreational drug, and increasingly appears as a pure compound or a component of 'herbal high' preparations in the Western world. While mitragynine/kratom may have analgesic, muscle relaxant and anti-inflammatory effects, its addictive properties and effects on cognitive performance are unknown. We isolated mitragynine from the plant and performed a thorough investigation of its behavioural effects in rats and mice. Here we describe an addictive profile and cognitive impairments of acute and chronic mitragynine administration, which closely resembles that of morphine. Acute mitragynine has complex effects on locomotor activity. Repeated administration induces locomotor sensitization, anxiolysis and conditioned place preference, enhances expression of dopamine transporter- and dopamine receptor-regulating factor mRNA in the mesencephalon. While there was no increase in spontaneous locomotor activity during withdrawal, animals showed hypersensitivity towards small challenging doses for up to 14 days. Severe somatic withdrawal signs developed after 12 hours, and increased level of anxiety became evident after 24 hours of withdrawal. Acute mitragynine independently impaired passive avoidance learning, memory consolidation and retrieval, possibly mediated by a disruption of cortical oscillatory activity, including the suppression of low-frequency rhythms (delta and theta) in the electrocorticogram. Chronic mitragynine administration led to impaired passive avoidance and object recognition learning. Altogether, these findings provide evidence for an addiction potential with cognitive impairments for mitragynine, which suggest its classification as a harmful drug.

Keywords Addiction, cognition, kratom, methamphetamine, mitragynine, morphine.

Correspondence to: Christian P. Müller, Section of Addiction Medicine, Department of Psychiatry and Psychotherapy, Friedrich-Alexander-University of Erlangen-Nuremberg, Schwabachanlage 6, Erlangen 91054, Germany. E-mail: Christian.Mueller@uk-erlangen.de

INTRODUCTION

Mitragyna speciosa Korth (*M. speciosa*) of the Rubiaceae (coffee) family is a medicinal herb indigenous to Malaysia and Thailand (Hassan *et al.* 2013). It is also known as *biak-biak* or *ketum* in Malaysia and *kratom*, *kakuam*, *kraton*, *ithang* or *thom* in Thailand (Jansen & Prast 1988; Ingsathit *et al.* 2009; Maruyama *et al.* 2009; Adkins, Boyer & McCurdy 2011). Preparations of its leaves are consumed by the native population in particular in rural areas (Vicknasingam *et al.* 2010). The dried leaves of *M. speciosa* can be chewed fresh, smoked or made into an extract (Hassan *et al.* 2013; Singh, Müller & Vicknasingam 2014). It is regularly used for its energizing and pain-relieving effects, which are reported to have psychostimulant- as well as opiate-like effects, depending on the dose consumed (Babu, McCurdy & Boyer 2008; Vicknasingam *et al.* 2010). The plant preparation is also used for opiate withdrawal and as substitution for the more expensive heroin (Vicknasingam *et al.* 2010; Ahmad & Aziz 2012). Easy availability of the plant allows for a wide spread and uncontrolled use with little understood health consequences (McWhirter & Morris 2010; Nelsen *et al.* 2010; Kapp *et al.* 2011). Withdrawal

*These authors contributed equally.

symptoms were reported to include hostility, aggression, aching of muscles and bones, jerky movements of the limbs, anorexia, weight loss, insomnia, and psychosis (Hassan *et al.* 2013; Singh *et al.* 2014).

The main psychoactive alkaloid of kratom is mitragynine, while the strongest pain relief is induced by the much less abundant 7-hydroxy-mitragynine (Matsumoto *et al.* 2005; Hassan *et al.* 2013). While herbal preparations of kratom are used either as a pure preparation or as an ingredient of 'legal-' or 'herbal high' preparations, which are distributed under various names, such as *krypton*, *K2* or *spice* (Dresen *et al.* 2010; Arndt *et al.* 2011; Logan *et al.* 2012), purified mitragynine becomes increasingly available on a worldwide scale via the Internet (Boyer *et al.* 2008; Hillebrand, Olszewski & Sedefov 2010). Emerging reports of *spice* and kratom use in the United States and Europe suggest a considerable abuse potential with adverse health effects (Boyer *et al.* 2008; Havemann-Reinecke 2011; Ward *et al.* 2011).

In Thailand, Malaysia and many European Union countries mitragynine and *M. speciosa* leaves or preparations are controlled drugs. In the United States, United Kingdom and Germany, they are currently not controlled substances, but are under surveillance awaiting more scientific evidence (EMCDDA/Kratom-Europa 2012; Hassan *et al.* 2013). Here we describe a systematic analysis of the abuse potential and cognitive impairments of mitragynine in rodent models.

MATERIAL AND METHODS

Animals and drugs

All experimental procedures were reviewed and approved by the Animal Ethics Committee, Universiti Sains Malaysia, Pulau Pinang, Malaysia or by the Regierung of Mittelfranken, Germany. Male Sprague-Dawley rats (Animal House, Universiti Sains Malaysia, Penang, Malaysia), weighing between 200 and 300 g at testing, served as subjects. For all experiments, new, experimentally naïve animals have been used.

Gene expression

Male C57BL/6 mice, 6 weeks old on their arrival (Charles River, Sulzfeld, Germany) were used. Morphine hydrochloride (Lipomed AG, Arlesheim, Switzerland) and d,l-methamphetamine hydrochloride (Lipomed AG) were used.

Mitragynine preparation

Mitragynine was extracted, isolated and verified from fresh leaves of *M. speciosa* as described previously (Utar *et al.* 2011). Fresh leaves of *M. speciosa* were obtained from Kedah and Perlis (Malaysia). The leaves were washed and oven dried at 40°C for 3 days. Finally, the dried leaves were grounded into powder. Dry, powdered leaves (300–400 g) were Soxhlet extracted using methanol at preset temperature of 40°C for 28 hours. The suspension was filtered and the methanol was removed by rotary evaporator to yield the crude methanolic extract. Subsequently, the methanol extract was dissolved in 10 percent (v/v) acetic acid solution, filtered and washed with hexane to form an acidic layer. The acidic layer was alkalinized to pH 9 using 25 percent (v/v) ammonia solution and extracted with dichloromethane. Finally, the extracts were evaporated by rotary evaporator to produce the alkaloid extract. Isolation of mitragynine from alkaloid extract was carried out using analytical and preparative high performance liquid chromatography (HPLC) mode using Waters Autopurification™ System with PDA Detector (Waters, Oslo, Norway). Chromatographic separation optimization was carried out in isocratic mode using a SunFireTM C18 column (4.6 × 250 mm, 5-µm particles), Waters. The isocratic system was used with a mobile phase consisting of methanol and acetate buffer (pH 6) at composition (75 percent v/v methanol/25 percent v/v acetate buffer). The flow rate was set at 0.8 ml/min. The injection volume was 20 µl with a 15-minute run time. The maximum concentration of alkaloid used was 8 mg/ml. PDA Detector was set from 200 to 300 nm. Subsequently, the optimized analytical HPLC condition was scaled-up to preparative HLPC mode for isolation of pure mitragynine with predetermined fraction collector settings. The mitragynine compound was obtained by pooling the fractions together and the solvent were evaporated using a rotary evaporator at 40°C. Purified mitragynine was confirmed by HPLC and hydrogen-1 nuclear magnetic resonance (400 MHz) analysis (Jamil *et al.* 2013). Mitragynine obtained by this procedure was approximately 98 percent pure. For testing, a dose range of 1–30 mg/kg mitragynine was used, with detailed doses adapted to respective experiments in pretests. Mitragynine was dissolved in 20 percent of Tween 80 as vehicle.

Locomotor activity

Rats were subjected to locomotor activity screening in an open field before treatment and 1 hour after injection on days 1, 7 and 14. Each test trial lasted for 30 minutes (Fernandes *et al.* 2012).

Anxiety measurements

The anxiety test was conducted using elevated plus maze (EPM) (Pum, Huston & Müller 2009) and the light dark box test (Crawley & Goodwin 1980) as described previously.

Conditioned place preference (CPP)

Preconditioning (days 1–2)

All rats received preconditioning test in the CPP paradigm box. Rats that exhibited unconditioned aversion (< 10 percent of the session) or preferences (> 60 percent of the session) for any chamber were discarded for conditioning sessions.

Conditioning (days 3–10)

On day 3, rats were randomly assigned to one of seven groups, each to receive either vehicle (Tween 80), mitragynine (1, 5, 10, 30 mg/kg), morphine (MOR; 10 mg/kg) or methamphetamine (METH; 1 mg/kg) during drug conditioning sessions ($n = 9–12$/group). Rats were assigned to receive vehicle or drug paired with one of the two conditioning chambers in a counterbalanced fashion (unbiased procedure).

CPP test (day 11)

Rats were placed into the central chamber with both doors open and were allowed free access to the entire apparatus for 20 minutes.

Locomotor sensitization and mRNA expression analysis

Mice were habituated to the testing procedure for 4 days before they were randomly assigned to one of three treatment groups: vehicle, 10 or 20 mg/kg mitragynine ($n = 15$/group). On the 1st, 4th, 7th and 10th day mice were placed into the open field after injections and locomotion was recorded for 20 minutes. Locomotor activity was automatically recorded using high-resolution infrared sensors. Animals were decapitated on the 11th day, the brain was removed, shock frozen in dry ice and immediately stored at −80°C. Then, ventral mesencephalon and ventral striatum were dissected and processed for analysis. The dopaminergic (DA) projection from the ventral tegmental area in the mesencephalon to the ventral striatum is a key projection mediating the reinforcing effects of addictive drugs (McBride, Murphy & Ikemoto 1999), and as such a first starting point for mechanistic analysis of mitragynine action in the brain's reward system. mRNA expression analysis was performed as described previously (De Souza Silva *et al.* 2013).

Withdrawal effects

Rats were treated daily with mitragynine [30 mg/kg; intraperitoneal (i.p.)] for 14 days. Locomotor activity was assessed on days 1, 7 and 14 for 30 minutes, 75 minutes after mitragynine administration. Somatic signs of spontaneous withdrawal were scored 12 and 72 hours after the last injection and then every 24 hours for 3 days. Withdrawal somatic signs were evaluated during a 30-minute period subdivided into 5-minute intervals (Castane *et al.* 2002). The total number of wet-dog shakes and fore paw tremors was counted. Body tremor, ptosis, teeth chattering, genital licks and piloerection were scored 1 for appearance or 0 for non-appearance, within each 5 minutes interval. 7, 14 and 21 days after the last dose of acute mitragynine or vehicle, rats were challenged with 3 mg/kg (i.p.) mitragynine and locomotor activity was recorded 75 minutes after challenge dose. Furthermore, anxiety levels were assessed 12, 24, 48 and 72 hours after mitragynine withdrawal in the EPM as described earlier.

Passive avoidance learning

The effects of mitragynine on passive avoidance learning were tested in a two-compartment shuttle box (Columbus Instruments, Columbus, OH, USA; Kart *et al.* 2004).

Effects on acquisition

Rats received mitragynine (1, 5, or 10 mg/kg), MOR (5 mg/kg) or vehicle 30 minutes prior to the training. On the test day, all animals received vehicle 30 minutes before the retention test ($n = 9–12$/group).

Effects on consolidation

New groups of rats received mitragynine (1, 5 or 10 mg/kg), MOR (5 mg/kg) or vehicle i.p. immediately after training (post-trial; $n = 9–12$/group).

Effects on memory retrieval

New groups of rats received vehicle prior to the training. On the test day, rats received mitragynine (1, 5 or 10 mg/kg), MOR (5 mg/kg) or vehicle (i.p.) 30 minutes before the retention test (pretest; $n = 9–12$/group).

Chronic study

New groups of rats received mitragynine (1, 5, or 10 mg/kg), MOR (5 mg/kg) or vehicle (i.p.) daily for 28 days. Animals received training on the first day of abstinence and a retention test was carried out 24 hours later. The retention test was repeated 3 and 7 days after the training session ($n = 9–12$/group).

Novel object recognition

Animals were habituated to the experimental apparatus for 10 minutes. Twenty-four hours later, animals were familiarized with two identical sample objects for 5 minutes. Immediately after this familiarization phase, five groups of animals received either vehicle, MOR (5 mg/kg) or mitragynine (1, 5 or 10 mg/kg). After 90 minutes, animals were tested for the object recognition for 3

minutes, in the presence of one familiar and one novel object (Bevins & Besheer 2006).

Electroencephalogram (EEG) activity

Six stainless-steel screws (Plastic One, Roanoke, VA, USA), implanted in a stereotaxic surgery over the hippocampus, sensory cortex or in the frontal cortex, were used as electrodes. The wireless head stage was plugged in on the experimental day. Each rat was placed in a Perspex cage (40 × 40 × 20 cm) 60 minutes before drugs administration. Rats receiving a single i.p. dose of either vehicle, METH (1 mg/kg), MOR (5 mg/kg) or mitragynine (1, 5 or 10 mg/kg; $n = 6$/group). The EEG, the electrocorticogram (ECoG) and locomotor activity were recorded simultaneously (Dringenberg *et al.* 2002).

Statistical analysis

All data were expressed as a mean ± standard error of the mean. Data were analysed by one- or two-way analysis of variance (ANOVA) followed by adjusted Fisher's least significant difference *post hoc* tests when appropriate. A significance level of $P < 0.05$ was used for statistical significance. The software used was GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA) and Statistica (StatSoft Inc., Tulsa, OK, USA). For more methodological and statistical details, see Supplement Information (SI).

RESULTS

Mitragynine effects on locomotor activity

It was reported that the drug might induce psychostimulant-like effects at low doses, and opiate-like effects at high doses (Hassan *et al.* 2013). Here we tested acute locomotor effects of mitragynine in the open field in rats. We found that mitragynine had a significant dose-dependent effect on locomotor activity ($F_{3,28} = 67.25$, $P < 0.001$; Fig. 1a) and rearing behaviour ($F_{3,28} = 59.88$, $P < 0.001$; Fig. 1b). A low dose of 1 mg/kg induced profound hyper-locomotion and rearing behaviour ($P < 0.01$) compared with vehicle group, while a medium dose of 10 mg/kg and high dose 30 mg/kg significantly reduce the total distance travelled ($P < 0.01$) and rearing ($P < 0.01$) compared with the lowest dose-treated group. These findings suggest a stimulant effect on locomotor activity, but only at a low dose.

Acute mitragynine has anxiolytic effects

We further tested acute effects of mitragynine on emotional behaviours in the light–dark box test in rats (Crawley & Goodwin 1980). Our data suggest a significant U-shaped dose-dependent effect of mitragynine measured as the time spent in the light compartment ($F_{3,28} = 19.21$, $P < 0.001$). A low dose (1 mg/kg; $P < 0.01$) and a high dose (30 mg/kg; $P < 0.01$), but not a medium dose of mitragynine (10 mg/kg; $P > 0.05$), increased the time spent in the light compartment, which suggests an acute anxiolytic effect (Fig. 1c). In order to confirm these findings in another paradigm, we used the EPM test (Pum *et al.* 2009). Mitragynine induced a U-shaped dose-dependent anxiolytic response in rats (Fig. 1d) measured as the time spent on the open arms of the EPM ($F_{3,28} = 31.71$, $P < 0.001$). *Post hoc* analysis revealed a significant increase in time in the 1 and 30 mg/kg mitragynine-treated groups ($P = 0.0008$), but not in the 10 mg/kg treated group ($P > 0.05$). The locomotor activity in this test was similar among treatment groups ($P > 0.05$). Accordingly, the difference of time spent in the open arms was not due to differences in locomotor activity in this test. Our data suggest that a low and a high, but not a medium dose of mitragynine induced an acute anxiolytic effect.

CPP and conditioned locomotion

In order to measure the potential rewarding properties of mitragynine, we tested it during the CPP (Huston *et al.* 2013). Rats that were conditioned with MOR (10 mg/kg) and METH (1 mg/kg) established a significant CPP for the drug-paired chamber (MOR: $P = 0.003$; METH: $P = 0.0128$; Fig. 2a). Mitragynine induced a significant CPP at doses of 10 mg/kg ($P = 0.0211$) and 30 mg/kg ($P = 0.0333$). The results of CPP test suggest dose-dependent rewarding properties of mitragynine, which resemble in its magnitude those of MOR and METH.

METH induced a constant hyperlocomotion during all conditioning sessions ($P < 0.001$; Fig. 2b). MOR did not have an effect on locomotor activity on first treatment, but significantly increased locomotion in all other trials ($P < 0.05$), thus developing a locomotor sensitization. Only the highest dose of mitragynine (30 mg/kg) showed a sensitization after the fourth trial of conditioning ($P < 0.01$). There was no difference between treatment groups in acute locomotion after pseudo-conditioning with saline ($P > 0.05$; Fig. 2c).

METH and MOR induced a significant conditioned locomotion during CPP testing (Fig. 2d). Mitragynine also induced significant conditioned locomotion at the highest dose tested (30 mg/kg, $P = 0.0209$). Altogether, these findings show that mitragynine induces a dose-dependent CPP together with conditioned locomotion indicating persistent reinforcing and locomotor activating effects of the drug (for more statistical details, see SI).

Locomotor sensitization and mRNA expression

A hallmark of psychostimulant drugs is the development of locomotor sensitization (Vanderschuren & Kalivas

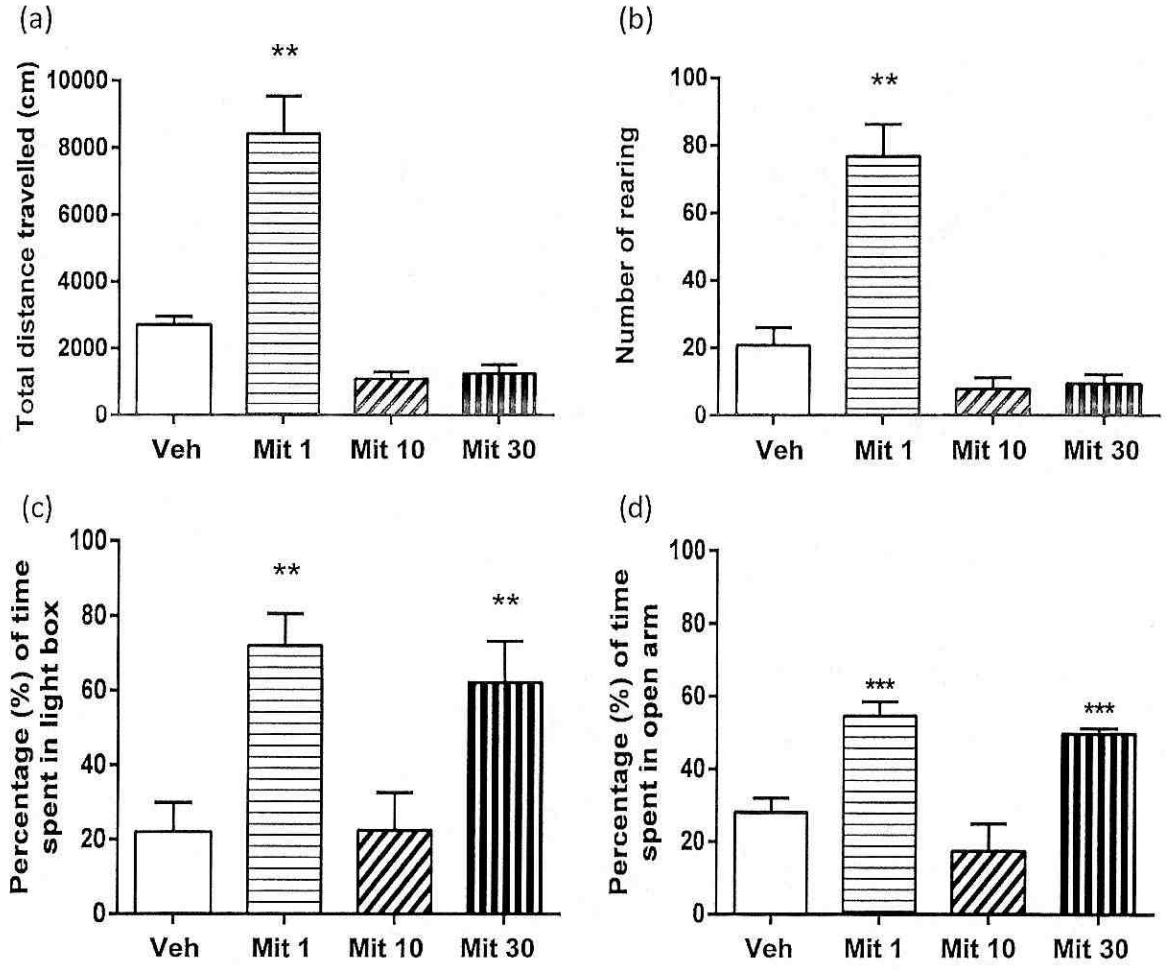

Figure 1 Acute mitragynine effects on locomotor activity and anxiety in rats. Effect of acute mitragynine (Mit) administration (1, 10 and 30 mg/kg; i.p.) on (a) locomotor activity and (b) rearing behaviour in the open field ($n = 8$/group). (c) Anxiolytic effects of Mit on anxiety-related behaviour in the light–dark box as shown by the percentage of time spent in the aversive light box versus total time in light and dark box. (d) Anxiolytic effects of Mit in the elevated plus maze test as shown by an increase in the percent time spent on the open arms versus overall arm time [mean ± standard error of the mean; $n = 8$/group; $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle (Veh)]

2000) and plasticity in the DA system (Volkow, Fowler & Wang 2002). We found that sub-chronic administration of mitragynine induced a sensitization of the locomotor-stimulating effect (Fig. 2e), which was similar in the mice as compared with the rats (Fig. 1b). Mitragynine treatment had a significant effect on the DA D2 mRNA expression in the mesencephalon ($F_{2,42} = 34.172$, $P < 0.0001$; Fig. 2f) and the ventral striatum ($F_{2,42} = 3.254$, $P = 0.0485$; Fig. 2g). Treatment with 10 mg/kg mitragynine decreased DA D2 mRNA expression in the mesencephalon ($P < 0.0001$). Dopamine clearance from the synapse is controlled by the dopamine transporter (DAT) (Kuhar & Pilotte 1996). Mitragynine had an effect on DAT mRNA expression in the mesencephalon ($F_{2,39} = 6.252$, $P = 0.0044$; Fig. 2h), but not in the ventral striatum ($P > 0.05$, Fig. 2i). There was a significant increase in DAT mRNA expression for the highest mitragynine dose tested ($P = 0.0046$). The dopamine receptor-regulating factor (DRRF) controls the expression of dopamine receptors in the brain (Hwang *et al.* 2001). We found an increase in DRRF mRNA expression in the mesencephalon ($F_{2,42} = 3.0997$, $P = 0.0555$; Fig. 2j), but not in the ventral striatum ($P > 0.05$; Fig. 2k) after 20 mg/kg mitragynine ($P = 0.0414$). These data suggest that mitragynine leads to a sensitization of acute locomotor response. Behavioural plasticity was accompanied by enhanced expression of DAT and DRRF mRNA in a brain region containing DA neurons, but not in the target areas of their projections.

Withdrawal is associated with the expression of behavioural sensitization

We tested rats that received mitragynine (30 mg/kg; i.p.) for 14 days and observed increased locomotor activity after withdrawal. In line with previous findings, two-way ANOVA revealed that mitragynine induced a sensitized

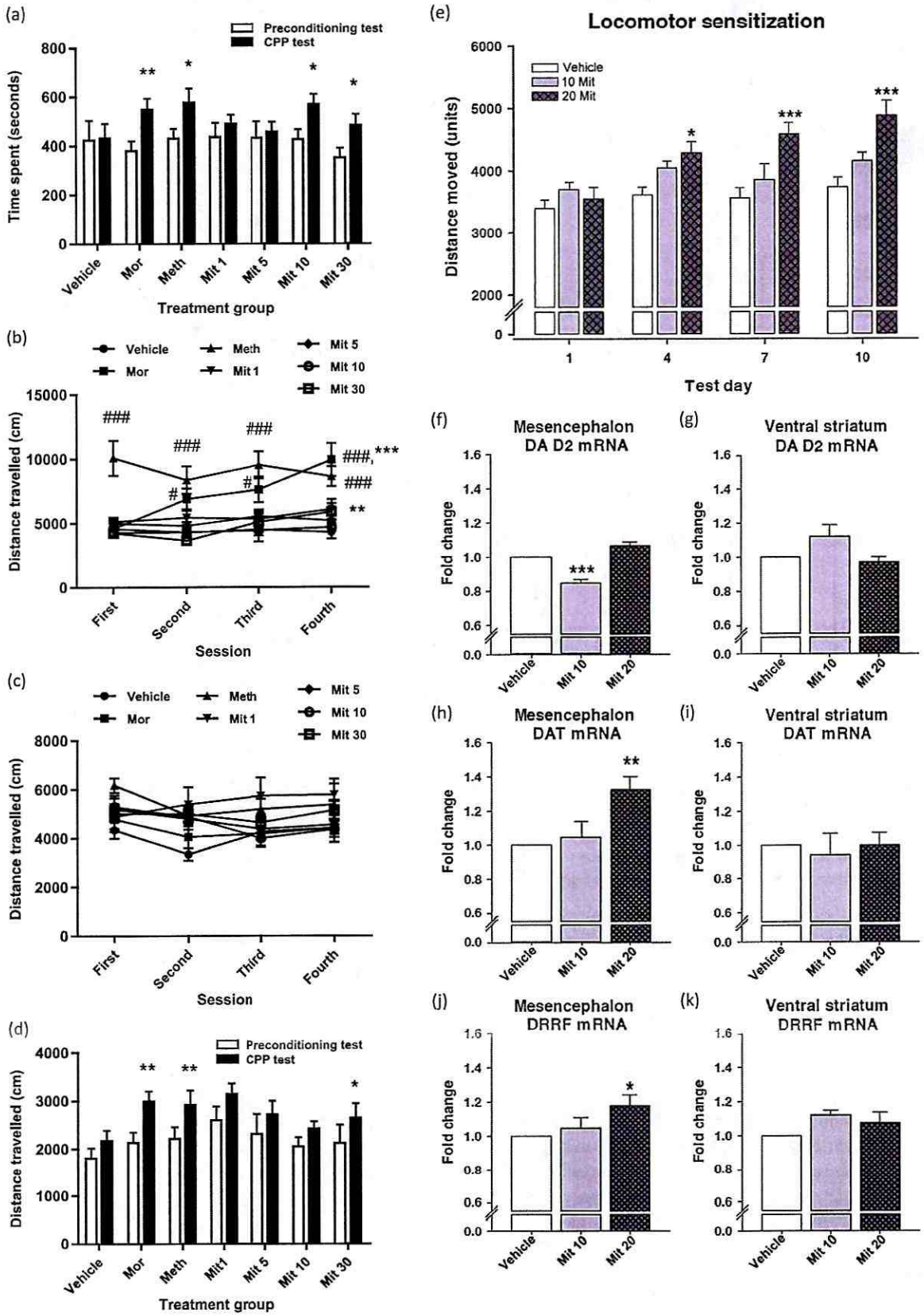

Figure 2 Mitragynine (Mit) has reinforcing effects and induces conditioned locomotor activity and locomotor sensitization in rats (mean ± standard error of the mean). (a) Drug-induced conditioned place preference after morphine (Mor; 10 mg/kg), methamphetamine (Meth; 1 mg/kg) and Mit (1, 5, 10, 30 mg/kg) as shown by the time spent in the conditioning compartment (*$P < 0.05$, **$P < 0.01$ versus preconditioning baseline; $n = 10$–12/group). Locomotor activity during, (b) the four conditioning trials with the drug, and (c) during the four pseudo-conditioning trials with vehicle ($^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ versus vehicle; **$P < 0.01$, ***$P < 0.001$ versus first treatment day). (d) Conditioned locomotor effect observed during drug-free conditioned place preference (CPP) testing (*$P < 0.05$, **$P < 0.01$ versus preconditioning baseline). (e) Dose-dependent sensitization of locomotor effects of Mit after 10 treatment trials with Mit (i.p.) in mice (*$P < 0.05$, ***$P < 0.001$ versus vehicle). (f–k) Repeated Mit treatment induces changes in mRNA expression of dopaminergic genes in the mesencephalon, but not in the ventral striatum. DA D2, dopamine D2 receptor; DAT, dopamine transporter; DRRF, dopamine receptor-regulating factor. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$ versus vehicle

locomotor activation ($F_{5,35} = 23.28$, $P < 0.0001$), which was significant after 7 and 14 ($P = 0.003$) days of treatment. There were no effects on locomotor activity during the subsequent 3 days of withdrawal ($P > 0.05$; Fig. 3a). However, when leaving animals undisturbed for 4 days and then challenging them with a small dose of mitragynine (3 mg/kg, i.p.), animals showed a hypersensitized locomotor response, which resembled that after sensitization to a high dose ($F_{2,14} = 58.79$, $P < 0.0001$; Fig. 3b). This response peaked after 7 and 14 days ($P < 0.0001$), but was reversed to control levels after 21 days ($P > 0.05$).

Withdrawal-induced anxiety

Withdrawal from psychostimulant and opioide drugs is associated with an aversive state and increased anxiety levels (Wood & Lal 1987). We measured behaviour in the EPM test of anxiety after withdrawal from a 14-day daily mitragynine treatment (30 mg/kg; i.p.) and found a significant increase in anxiety levels as indicated by the time animals spent on open arms ($F_{3,21} = 25.72$, $P < 0.0001$; Fig. 3c). Our data showed no effects on anxiety after 12 hours. However, after 24 hours ($P = 0.00104$) and 48 hours ($P = 0.0002$), there was a significant decline in open arm time. This effect was reversed after 72 hours ($P > 0.05$), suggesting a restricted period of withdrawal-induced anxiety.

Withdrawal signs after mitragynine cessation

Behavioural withdrawal symptoms have been reported after cessation of chronic opiate and psychostimulant use in humans and in animal models. We found that mitragynine withdrawal induced a paw tremor, body tremor, wet-dog shakes, ptosis, piloerection, teeth chattering and grooming 12 hours after withdrawal (Table 1). Except paw tremor and grooming, all effects were still present 48 hours after withdrawal. Most of them disappeared after 72 hours. These findings suggest profound withdrawal effects in the first 48 hours after cessation of chronic mitragynine treatment.

Impaired learning and memory after acute mitragynine

In order to investigate the effects of acute and chronic mitragynine exposure on cognition, we measured the effects of mitragynine in a passive avoidance task in rats and compared them with MOR (5 mg/kg, i.p.). Pretraining administration of mitragynine (1, 5 or 10 mg/kg, i.p.; $P < 0.01$ and $P < 0.001$ versus vehicle) significantly reduced the step-through latency in the retention test ($F_{4,45} = 7.1$, $P = 0.0002$). These reductions in step-through latency were similar to the extent of reduction in the MOR-treated group ($P < 0.01$; Fig. 4a).

Then we asked whether mitragynine would impair memory consolidation in the passive avoidance task. A new batch of animals received mitragynine or MOR in the post-training consolidation phase (Fig. 4b). Mitragynine dose-dependently impaired memory consolidation in a passive avoidance task to a similar degree as MOR ($F_{4,55} = 5.581$, $P = 0.0008$; $P < 0.01$ and $P < 0.001$ versus vehicle).

The consumption of addictive drugs may also impair the retrieval of already established memories. In order to test whether mitragynine affects retrieval, new animals were trained in a passive avoidance task (Fig. 4c). They received mitragynine or MOR only before retrieval testing. Mitragynine impaired the retrieval of a passive avoidance task in rats ($F_{4,40} = 5.322$, $P = 0.0016$; $P < 0.01$ versus vehicle). This impairment was again comparable with the one induced by MOR ($P < 0.05$). Altogether, these results show that acute mitragynine has profound cognition-impairing effects already after a small dose, which independently affected learning, consolidation and retrieval of a new task.

Chronic mitragynine induces learning impairments

Chronically used psychoactive drugs may have long-lasting negative effects on cognition during abstinence. We treated rats for 28 days with mitragynine (1, 5 or 10 mg/kg, i.p) or MOR (5 mg/kg, i.p.). Animals were then trained in a passive avoidance task and tested during abstinence (Fig. 4d). Our results show a significant impairment of learning and memory after all doses tested

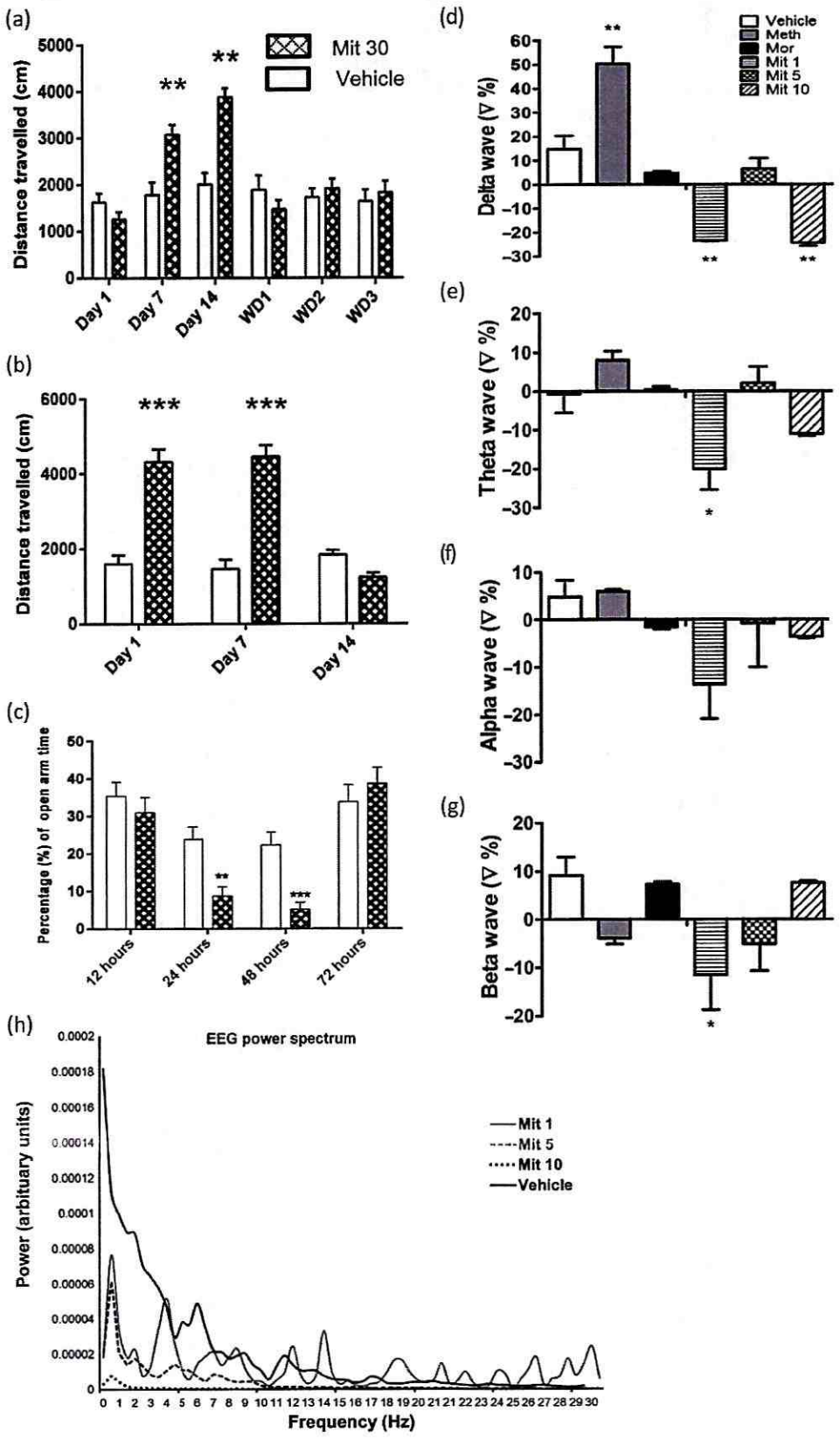

Figure 3 Withdrawal from chronic mitragynine (Mit) induces acute anxiety and is associated with sensitized hyperactivity. (a) Locomotor activity during a 14-day Mit treatment (tested on days 1, 7 and 14) and during early withdrawal days (WD) 1–3. (b) Sensitized locomotor activation after a small dose of Mit (3 mg/kg, i.p) during late withdrawal. (c) Anxiogenic effects of Mit withdrawal in the elevated plus maze test as shown by an increase in the percentage of time spent on the open arms versus overall arm time [mean ± standard error of the mean (SEM); $n = 8$/group; $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle]. Effects of Mit, morphine (Mor) and methamphetamine (Meth) on the hippocampus electroencephalogram (EEG) in freely moving rats. Percentage difference of the average cyclic height (mV) in the (d) delta, (e) theta, (f) alpha and (g) beta bands (mean ± SEM; $n = 6$/group; $^{*}P < 0.05$, $^{**}P < 0.01$ versus vehicle). (h) The effect of Mit on frontal cortex electrocorticogram. Power spectral analyses for 10 seconds epochs recorded 30 minutes after drug administration ($n = 5$–6/group)

on days 1 ($F_{4,50} = 4.4$, $P = 0.004$), 3 ($F_{4,50} = 19.65$, $P < 0.0001$) and 7 ($F_{4,50} = 29.03$, $P < 0.0001$) of withdrawal. These effects were comparable with the impairment induced by MOR ($P < 0.05$).

We further tested novel object recognition in the same chronically treated animals after 10 days of abstinence (Dere, Huston & De Souza Silva 2007). The ability to recognize the novel object was impaired after MOR ($P = 0.0432$; Fig. 4e). Mitragynine had a dose-dependent memory-impairing effect, which was significant at the highest dose tested (10 mg/kg; $P = 0.0174$). These results of discrimination ratio were paralleled by the number of direct contacts with familiar and novel objects in each treatment group (Fig. 4f; for more statistical details, see SI). Present findings suggest that chronic mitragynine treatment can induce learning and memory impairment during abstinence.

Suppressed EEG/ECoG activity after mitragynine

In order to explore neuronal mechanisms that underlie the cognitive deficits induced by mitragynine, we measured effects of mitragynine (1, 5, 10 mg/kg, i.p.) on EEG activity in the hippocampus and sensory cortex in freely moving animals (Dringenberg & Vanderwolf 1998; Dimpfel 2008). Single-dose administration of METH (1 mg/kg) led to an increase in locomotion (Supporting Information Fig. S2), and in large amplitude low-frequency rhythms in the EEG delta band of the hippocampus ($P < 0.01$; Fig. 3d–g). The 1-mg/kg mitragynine dose produced a significant decrease of activity in the delta, theta and beta, but not alpha band in the hippocampus (*delta*: $F_{5,11} = 24.87$, $P < 0.0001$; *theta*: $F_{5,11} = 5.215$, $P = 0.0107$; *beta*: $F_{5,11} = 4.450$, $P = 0.0184$; *alpha*: $P > 0.05$; *delta*: $P < 0.01$, *theta* and *beta*: $P < 0.05$ versus vehicle), while a dose of 5 mg/kg had no effect ($P > 0.05$). A dose of 10 mg/kg, however, led again to a significant decrease in the delta band in the hippocampus ($P < 0.01$). A suppression of brain activity was also seen in the sensory cortex, but did not reach statistical significance (Supporting Information Fig. S3).

In addition, we performed an analysis of the ECoG recorded from the frontal cortex (Fig. 3h), which revealed that the ECoG was dominated by prominent peaks in the power spectrum of less than 10 Hz, i.e. by delta (0.1–3.5 Hz) and theta (3.6–7.5 Hz) bands. Administration of mitragynine dose-dependently suppressed delta and theta activities ($F_{3,120} = 8.196$, $P < 0.0001$). These data suggest that mitragynine predominantly suppresses EEG delta power in the hippocampus and the ECoG delta and theta power in the frontal cortex.

DISCUSSION

Here we present a thorough characterization of the addiction-related and cognitive-impairing effects of acute and chronic mitragynine in animal models. This study shows that acute mitragynine induces acute locomotor activation at a low dose in rats, but not at high doses in rats and mice (Hazim, Mustapha & Mansor 2011). Thereby, an increase was not observed within the first 20 minutes after administration, but within 1 hour in a familiar environment. Acute administration reduces anxiety in two different tests in this study, which is in line with previous reports of acute anxiolytic and antidepressant action (Farah *et al.* 2011). Mitragynine induces a CPP, conditioned locomotion and a sensitization of the locomotor-stimulating effects, which are well-known addiction-related drug effects in animal models. The behavioural effects of mitragynine show at the highest dose tested a similar magnitude as the effects of the opioid and psychostimulant drugs, MOR and METH. However, acute locomotor effects in a CPP environment appeared less pronounced. This may be due to an interaction with the emotional load of an open field or EPM and a CPP test environment. Mitragynine showed different dose–response curves for locomotor, anxiolytic and rewarding effects. This is well known for psychostimulant-type drugs (Müller *et al.* 2007). The establishment of behavioural plasticity goes along with a sensitization of the DA system in the mesencephalon, but not in the ventral striatum, reflected by an enhanced expression of DAT and DRRF mRNA. Withdrawal from chronic mitragynine induces opioid-like somatic withdrawal, locomotor hypersensitivity for further pharmacological stimulation and enhances anxiety levels for up to 48 hours. This is in line with reports in humans that describe hostility, aggression, aching of muscles and bones, jerky movements of the limbs, and anorexia,

Table 1 Somatic signs of spontaneous mitragynine withdrawal in animals repeatedly treated with saline and mitragynine during the 4 observation sessions at 12, 24, 48 and 72 hours after withdrawal.

	Withdrawal period (hours)									
	12		*24*		*48*		*72*		*One-way ANOVA*	
Sign	*Vehicle*	*Mit 30*	*Vehicle*	*Mit 30*	*Vehicle*	*Mit 30*	*Vehicle*	*Mit 30*	*F(4.35)*	*P <*
Paw tremor	2.15 ± 0.3	5.50 ± 0.8**	1.60 ± 0.8	6.25 ± 0.8**	2.12 ± 0.5	2.75 ± 0.4	2.40 ± 0.4	2.50 ± 1.2	12.8	0.001
Body tremor	0.75 ± 0.5	3.50 ± 0.5**	0.50 ± 0.2	5.40 ± 0.5**	1.25 ± 0.2	2.00 ± 0.5**	0.75 ± 0.2	1.38 ± 0.2	34.36	0.001
Wet-dog shakes	0.25 ± 1.9	3.60 ± 0.4**	0.38 ± 0.2	9.60 ± 0.8**	0.75 ± 0.4	5.50 ± 0.9**	0.75 ± 0.4	1.80 ± 0.4	63.09	0.001
Genital licking	0.50 ± 0.4	1.40 ± 0.3	0.63 ± 0.2	0.75 ± 0.4	0.75 ± 0.3	0.75 ± 0.3	0.50 ± 0.3	0.50 ± 0.4	2.17	0.09
Ptosis	0.25 ± 0.2	3.90 ± 0.4**	0.38 ± 0.2	5.75 ± 0.2**	0.38 ± 0.5	1.60 ± 0.2**	0.50 ± 0.2	1.38 ± 0.5*	82.91	0.001
Piloerection	0.50 ± 0.2	3.80 ± 0.4**	0.36 ± 0.2	3.40 ± 0.5**	0.63 ± 0.2	0.50 ± 0.3*	0.50 ± 0.2	1.00 ± 0.3	33.43	0.001
Teeth chattering	0.63 ± 0.5	3.00 ± 0.6**	0.50 ± 0.3	4.75 ± 0.5**	0.37 ± 0.2	2.25 ± 1.9*	0.37 ± 0.2	1.40 ± 0.4	21.6	0.001
Grooming	0.87 ± 0.3	3.50 ± 0.8**	0.63 ± 0.2	4.50 ± 0.6**	1.00 ± 0.4	1.75 ± 0.4	1.00 ± 0.4	1.50 ± 0.3	17.66	0.001

*$P < 0.05$; **$P < 0.01$ versus vehicle.

insomnia and psychosis as withdrawal symptoms (Sheleg & Collins 2011; Singh *et al.* 2014). Altogether, the behavioural profile suggests that mitragynine has the properties of an addictive drug in various preclinical tests. Although euphoric effects are less pronounced than those of MOR or METH, mitragynine may have a comparable harm potential (Nutt *et al.* 2007).

Besides its potentially addictive effects, acute as well as chronic mitragynine treatment caused profound impairments of learning and memory. In our study, mitragynine impaired the learning, consolidation and retrieval of a passive avoidance task already at low doses. This effect was of similar magnitude as the effects of MOR and METH. Also the withdrawal from chronic mitragynine caused deficits in learning and memory as seen during early withdrawal and during abstinence. While during early withdrawal, this effect was also very sensitive to low doses, late withdrawal deficits required a higher dose of chronic treatments. An analysis of the mitragynine effects on the EEG revealed a significant decline of delta power in the hippocampus and a complete suppression of the delta and theta bands in the ECoG of the frontal cortex as potential mechanisms for acute impairments in learning and memory. These findings suggest that acute as well as chronic mitragynine can cause profound cognitive impairments, which are still evident during withdrawal and can last for at least 10 days of abstinence.

While for addictive as well as cognitive-impairing effects, potential mechanisms had been identified, and it should be noted that they are probably not exhaustive. Previous studies suggest that mitragynine acts by binding to μ-, δ- and κ-opioid receptors (Tohda *et al.* 1997; Raffa *et al.* 2013; Stolt *et al.* 2014), which is in line with the reported analgesic effect (Takayama *et al.* 2002), but may also drive opiate-like rewarding effects in the brain. Also serotonergic mechanisms of mitragynine action have been described (Kumarnsit *et al.* 2007), which may contribute to the psychostimulant-like effects (Müller *et al.* 2007) and warrant further investigation of mitragynine effects. It is also important to acknowledge that there are other psychoactive compounds in kratom preparations that may well contribute to the analgesic and psychoactive effects (Hassan *et al.* 2013; Stolt *et al.* 2014).

In summary, the present result from rodent models clearly suggest that mitragynine, the main alkaloid of the psychoactive herb kratom/ketum, has a significant abuse and addiction potential and can cause profound emotional and cognitive impairments which resemble that of opiate and psychostimulant drugs.

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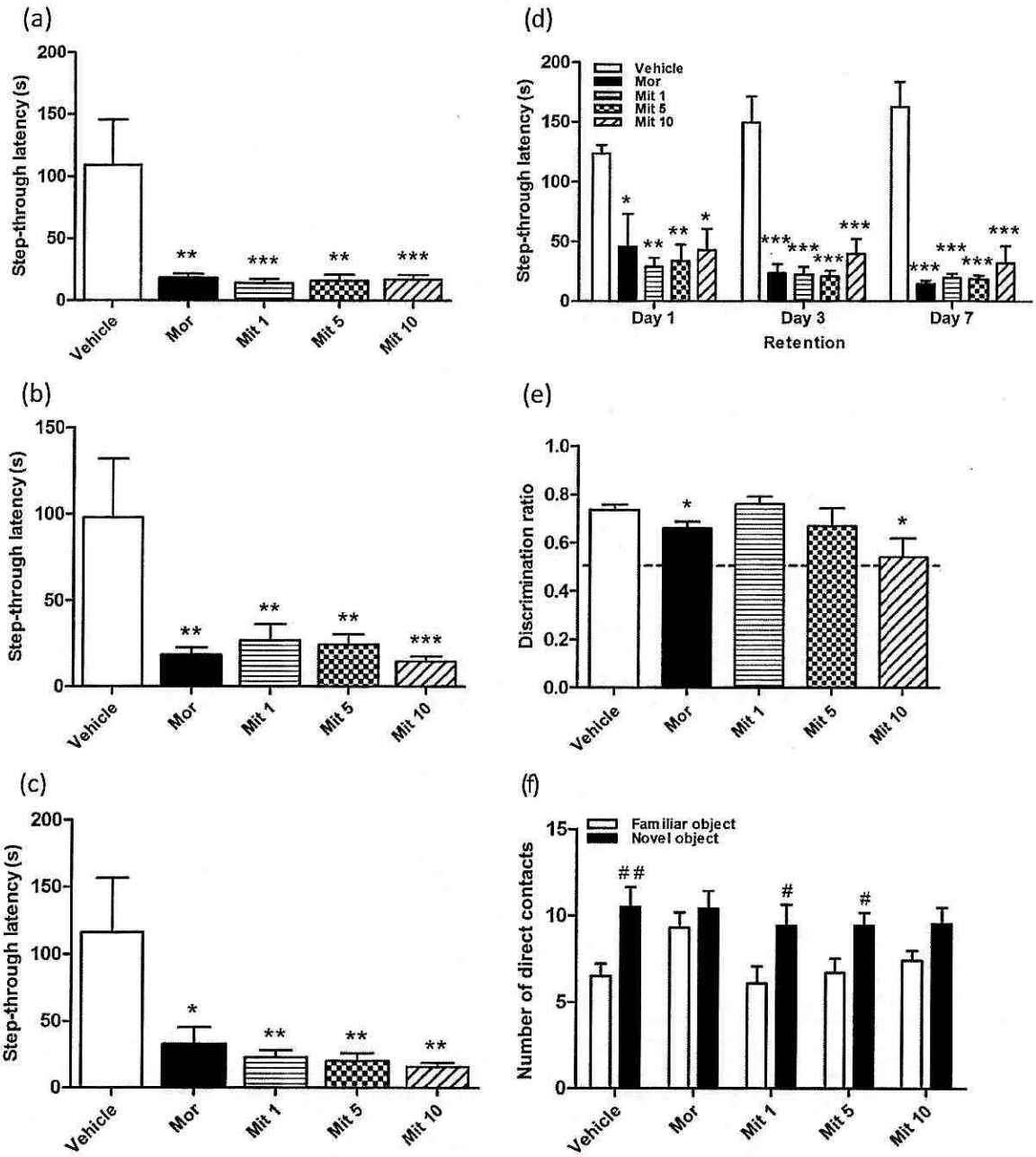

Figure 4 Learning and memory impairments after acute and chronic mitragynine (Mit) treatment. The effects of Mit (1, 5, 10 mg/kg; i.p.) and morphine (Mor; 5 mg/kg, i.p.) on step-through latencies [mean ± standard error of the mean (SEM)] during (a) learning (administered pre-trial), (b) consolidation (administered post-trial), and (c) retrieval (administered pretesting) of a passive avoidance task in rats. (d) Effects of chronic 28-day administration of Mit and Mor on learning and memory in a passive avoidance task during withdrawal. (e) Learning impairments during withdrawal from 28 days of chronic administration of Mit or Mor measured as discrimination ratio in an object recognition task in rats. (f) Direct contact with objects in object recognition task during retrieval testing (mean ± SEM; $n = 9$–12/group in each experiment; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus vehicle; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus novel object within group)

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Authors Contribution

CPM, VN and ZH designed the study. NHMY, FWS, RKV, ZH and ARü performed the experiments. NHMY, FWS, RKV, ZH, ARü and CPM analysed the data. DA, ARo, HCD

and SMM assisted with data acquisition and analysis. CPM was responsible for drafting the paper. All authors critically reviewed content and approved the final version for publication.

References

Adkins JE, Boyer EW, McCurdy CR (2011) *Mitragyna speciosa*, a psychoactive tree from Southeast Asia with opioid activity. Curr Top Med Chem 11:1165–1175.

Ahmad K, Aziz Z (2012) *Mitragyna speciosa* use in the northern states of Malaysia: a cross-sectional study. J Ethnopharmacol 141:446–450.

Arndt T, Claussen U, Güssregen B, Schröfel S, Stürzer B, Werle A, Wolf G (2011) Kratom alkaloids and O-desmethyltramadol in urine of a 'krypton' herbal mixture consumer. Forensic Sci Int 208:47–52.

Babu KM, McCurdy CR, Boyer EW (2008) Opioid receptors and legal highs: *Salvia divinorum* and kratom. Clin Toxicol (Phila) 46:146–152.

Bevins RA, Besheer J (2006) Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study 'recognition memory'. Nat Protoc 1:1306–1311.

Boyer EW, Babu KM, Adkins JE, McCurdy CR, Halpern JH (2008) Self-treatment of opioid withdrawal using kratom (*Mitragyna speciosa* Korth.). Addiction 103:1048–1050.

Castane A, Valjent E, Ledent C, Parmentier M, Maldonado R, Valverde O (2002) Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence. Neuropharmacology 43:857–867.

Crawley J, Goodwin FK (1980) Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 13:167–170.

De Souza Silva MA, Lenz B, Rotter A, Biermann T, Peters O, Ramirez A, Jessen F, Maier W, Hüll M, Schröder J, Frölich L, Teipel S, Gruber O, Kornhuber J, Huston JP, Müller CP, Schäble S (2013) Neurokinin3 receptor as a target to predict and improve learning and memory in the aged organism. Proc Natl Acad Sci USA 110:15097–15102.

Dere E, Huston JP, De Souza Silva MA (2007) The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. Neurosci Biobehav Rev 31:673–704.

Dimpfel W (2008) Pharmacological modulation of dopaminergic brain activity and its reflection in spectral frequencies of the rat electropharmacogram. Neuropsychobiology 58:178–186.

Dresen S, Ferreirós N, Pütz M, Westphal F, Zimmermann R, Auwärter V (2010) Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. J Mass Spectrom 45:1186–1194.

Dringenberg HC, Vanderwolf CH (1998) Involvement of direct and indirect pathways in electrocorticographic activation. Neurosci Biobehav Rev 22:243–257.

Dringenberg HC, Rubenstein ML, Solty H, Tomaszek S, Bruce A (2002) Electroencephalographic activation by tacrine, deprenyl, and quipazine: cholinergic versus non-cholinergic contributions. Eur J Pharmacol 447:43–50.

EMCDDA/Kratom-Europa (2012) Kratom *(Mitragyna speciosa)*. Available at: http://www.emcdda.europa.eu/publications/drug-profiles/kratom/de. Accessed 10 June 2012.

Farah IN, Taufik HM, Moklas MA, Sharida F, Raudzah AR, Shamima AR, Apryani E (2011) Antidepressant-like effect of mitragunine isolated from *Mitragyna speciosa* Korth in mice model of depression. Phytomedicine 18:402–407.

Fernandes AR, Easton AC, De Souza Silva MA, Schumann G, Müller CP, Desrivières S (2012) Lentiviral-mediated gene delivery reveals distinct roles of nucleus accumbens dopamine D2 and D3 receptors in novelty- and light-induced locomotor activity. Eur J Neurosci 35:1344–1353.

Hassan Z, Muzaimi M, Navaratnam V, Yusoff NH, Suhaimi FW, Vadivelu R, Vicknasingam BK, Amato D, von Hörsten HS, Ismail NI, Jayabalan N, Hazim AI, Mansor SM, Müller CP (2013) From kratom to mitragynine and its derivatives: physiological and behavioural effects related to use, abuse, and addiction. Neurosci Biobehav Rev 37:138–151.

Havemann-Reinecke U (2011) Kratom and alcohol dependence: clinical symptoms, withdrawal treatment and pharmacological mechanisms—a case report. Eur Psychiatry 26 (Suppl. 1):P1–P50.

Hazim AI, Mustapha M, Mansor SM (2011) The effects on motor behaviour and short-term memory tasks in mice following an acute administration of *Mitragyna speciosa* alkaloid extract and mitragynine. J Med Plants Res 5:5810–5817.

Hillebrand J, Olszewski D, Sedefov R (2010) Legal highs on the internet. Subst Use Misuse 45:330–340.

Huston JP, Silva MA, Topic B, Müller CP (2013) What's conditioned in conditioned place preference? Trends Pharmacol Sci 34:162–166.

Hwang CK, D'Souza UM, Eisch AJ, Yajima S, Lammers CH, Yang Y, Lee SH, Kim YM, Nestler EJ, Mouradian MM (2001) Dopamine receptor regulating factor, DRRF: a zinc finger transcription factor. Proc Natl Acad Sci USA 98:7558–7563.

Ingsathit A, Woratanarat P, Anukarahanonta T, Rattanasiri S, Chatchaipun P, Wattayakorn K, Lim S, Suriyawongpaisal P (2009) Prevalence of psychoactive drug use among drivers in Thailand: a roadside survey. Accid Anal Prev 41:474–478.

Jamil MF, Subki MF, Lan TM, Majid MI, Adenan MI (2013) The effect of mitragynine on cAMP formation and mRNA expression of mu-opioid receptors mediated by chronic morphine treatment in SK-N-SH neuroblastoma cell. J Ethnopharmacol 148:135–143.

Jansen KLR, Prast C (1988) Ethnopharmacology of kratom and the Mitragyna alkaloids. J Ethnopharmacol 23:115–119.

Kapp FG, Maurer HH, Auwärter V, Winkelmann M, Hermanns-Clausen M (2011) Intrahepatic cholestasis following abuse of powdered kratom (*Mitragyna speciosa*). J Med Toxicol 7:227–231.

Kart E, Jocham G, Müller CP, Schlömer C, Brandão ML, Huston JP, de Souza Silva MA (2004) Neurokinin-1 receptor antagonism by SR140333: enhanced *in vivo* ACh in the hippocampus and promnestic post-trial effects. Peptides 25:1959–1969.

Kuhar MJ, Pilotte NS (1996) Neurochemical changes in cocaine withdrawal. Trends Pharmacol Sci 17:260–264.

Kumarnsit E, Vongvatcharanon U, Keawpradub N, Intasaro P (2007) Fos-like immunoreactivity in rat dorsal raphe nuclei induced by alkaloid extract of *Mitragyna speciosa*. Neurosci Lett 416:128–132.

Logan BK, Reinhold LE, Xu A, Diamond FX (2012) Identification of synthetic cannabinoids in herbal incense blends in the United States. J Forensic Sci 57:1168–1180.

Maruyama T, Kawamura M, Kikura-Hanajiri R, Takayama H, Goda Y (2009) The botanical origin of kratom (*Mitragyna speciosa*; Rubiaceae) available as abused drugs in the Japanese markets. J Nat Med 63:340–344.

Matsumoto K, Horie S, Takayama H, Ishikawa H, Aimi N, Ponglux D, Murayama T, Watanabe K (2005) Antinociception, tolerance and withdrawal symptoms

induced by 7-hydroxymitragynine, an alkaloid from the Thai medicinal herb *Mitragyna speciosa*. Life Sci 78:2–7.

McBride WJ, Murphy JM, Ikemoto S (1999) Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behav Brain Res 101:129–152.

McWhirter L, Morris S (2010) A case report if inpatient detoxification after kratom (*Mitragyna speciosa*) dependence. Eur Addict Res 16:229–231.

Müller CP, Carey RJ, Huston JP, De Souza Silva MA (2007) Serotonin and psychostimulant addiction: focus on 5-HT1A-receptors. Prog Neurobiol 81:133–178.

Nelsen JL, Lapoint J, Hodgman MJ, Aldous KM (2010) Seizure and coma following kratom (*Mitragyna speciosa* Korth) exposure. J Med Toxicol 6:424–426.

Nutt D, King LA, Saulsbury W, Blakemore C (2007) Development of a rational scale to assess the harm of drugs of potential misuse. Lancet 369:1047–1053.

Pum ME, Huston JP, Müller CP (2009) The role of cortical serotonin in anxiety and locomotor activity in Wistar rats. Behav Neurosci 123:449–454.

Raffa RB, Beckett JR, Brahmbhatt VN, Ebinger TM, Fabian CA, Nixon JR, Orlando ST, Rana CA, Tejani AH, Tomazic RJ (2013) Orally active opioid compounds from a non-poppy source. J Med Chem 56:4840–4848.

Sheleg SV, Collins GB (2011) A coincidence of addiction to 'kratom' and severe primary hypothyroidism. J Addict Med 5:300–301.

Singh D, Müller CP, Vicknasingam BK (2014) Kratom (*Mitragyna speciosa*) dependence, withdrawal symptoms and craving in regular users. Drug Alcohol Depend 139:132–137.

Stolt AC, Schröder H, Neurath H, Grecksch G, Höllt V, Meyer MR, Maurer HH, Ziebolz N, Havemann-Reinecke U, Becker A (2014) Behavioral and neurochemical characterization of kratom (*Mitragyna speciosa*) extract. Psychopharmacology (Berl) 231:13–25.

Takayama H, Ishikawa H, Kurihara M, Kitajima M, Aimi N, Ponglux D, Koyama F, Matsumoto K, Moriyama T, Yamamoto LT, Watanabe K, Murayama T, Horie S (2002) Studies on the synthesis and opioid agonistic activities of mitragynine-related indole alkaloids: discovery of opioid agonists structurally different from other opioid ligands. J Med Chem 45:1949–1956.

Tohda M, Thongpraditchote S, Matsumoto K, Murakami Y, Sakai S, Aimi N, Takayama H, Tongroach P, Watanabe H (1997) Effects of mitragynine on cAMP formation mediated by delta-opiate receptors in NG108-15 cells. Biol Pharm Bull 4:338–340.

Utar Z, Majid MIA, Adenan MI, Jamil MFA, Lan TM (2011) Mitragynine inhibits the COX-2 mRNA expression and prostaglandin E2 production induced by lipopolysaccharide in RAW264.7 macrophage cells. J Ethnopharmacol 136:75–82.

Vanderschuren LJ, Kalivas PW (2000) Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl) 151:99–120.

Vicknasingam B, Narayanan S, Beng GT, Mansor SM (2010) The informal use of ketum (*Mitragyna speciosa*) for opioid withdrawal in the northern states of peninsular Malaysia and implications for drug substitution therapy. Int J Drug Policy 21:283–288.

Volkow ND, Fowler JS, Wang GJ (2002) Role of dopamine in drug reinforcement and addiction in humans: results from imaging studies. Behav Pharmacol 13:355–366.

Ward J, Rosenbaum C, Hernon C, McCurdy CR, Boyer EW (2011) Herbal medicines for the management of opioid addiction: safe and effective alternatives to conventional pharmacotherapy? CNS Drugs 25:999–1007.

Wood DM, Lal H (1987) Anxiogenic properties of cocaine withdrawal. Life Sci 41:1431–1436.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 A locomotor sensitizing regime of daily mitragynine (Mit; 10 and 20 mg/kg; i.p.) did not affect body weight gain in rats over 11 days of treatment

Figure S2 Effect of drugs on spontaneous locomotor activity during electroencephalogram (EEG) recordings in freely moving rats. Each value represents the mean ± standard error of the mean (SEM) for six rats. Locomotor activity was observed for 60 minutes after drugs administration ($^*P < 0.05$ versus vehicle)

Figure S3 Effects of mitragynine, morphine and methamphetamine on the sensory cortex electroencephalogram (EEG) in freely moving rats. Percentage difference of the average cyclic height (mV) in the (a) delta, (b) theta, (c) alpha, and (d) beta

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Kratom (*Mitragyna speciosa*) dependence, withdrawal symptoms and craving in regular users

Darshan Singh[a], Christian P. Müller[b,*], Balasingam K. Vicknasingam[a]

[a] Centre for Drug Research, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
[b] Department of Psychiatry and Psychotherapy, University Clinic, Friedrich-Alexander-University of Erlangen-Nuremberg, Schwabachanlage 6, 91054 Erlangen, Germany

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ABSTRACT

Background: Kratom (*Mitragyna speciosa*) preparations have been traditionally used in Southeast Asia for its medicinal properties. Lately, Kratom use has spread to Europe and the US, where abuse potential and health hazards increasingly emerge. This study is the first to measure systematically Kratom dependence, withdrawal symptoms, and drug craving in regular Kratom users in Malaysia.
Methods: A cross-sectional survey of 293 regular Kratom users was conducted in the community across three northern peninsular states of Malaysia. The Leeds Dependence Questionnaire, Marijuana Withdrawal Checklist, and Marijuana Craving Questionnaire-Short Form were used to measure Kratom dependence, withdrawal and craving.
Results: More than half of the regular users (>6 month of use) developed severe Kratom dependence problems, while 45% showed a moderate Kratom dependence. Physical withdrawal symptoms commonly experienced include muscle spasms and pain, sleeping difficulty, watery eyes/nose, hot flashes, fever, decreased appetite, and diarrhoea. Psychological withdrawal symptoms commonly reported were restlessness, tension, anger, sadness, and nervousness. The average amount of the psychoactive compound, mitragynine, in a single dose of a Kratom drink was 79 mg, suggesting an average daily intake of 276.5 mg. Regular users who consumed ≥3 glasses Kratom per day, had higher odds of developing severe Kratom dependence, withdrawal symptoms, and inability to control Kratom craving.
Conclusions: The findings from this study show that regular Kratom use is associated with drug dependency, development of withdrawal symptoms, and craving. These symptoms become more severe with prolonged use and suggest a stronger control of the drug.

1. Introduction

Kratom, also known as Ketum or '*biak*', is an indigenous plant (*Mitragyna speciosa* Korth) of Southeast Asia. Kratom has gained widespread popularity as a folk remedy among the rural population of Malaysia and Thailand (Adkins et al., 2011; Hassan et al., 2013). People use Kratom leaves for their energising and pain relieving effects which are reported to have psychostimulant-as well as opiate-like character, depending on the dose consumed (Babu et al., 2008; Vicknasingam et al., 2010). The plant preparation is also used for opiate withdrawal and as substitution for the more expensive heroin (Suwanlert, 1975; Assanangkornchai et al., 2007a,b; Vicknasingam et al., 2010; Ahmad and Aziz, 2012).

The main psychoactive alkaloid of Kratom is mitragynine while the strongest pain relieve is induced by the less abundant 7-hydroxy-mitragynine (Matsumoto et al., 2005; Hassan et al., 2013). Herbal preparations of Kratom are increasingly found and used in Europe and the US, either as pure preparation (Cornara et al., 2013; Forrester, 2013) or as one herbal ingredient of 'legal-' or 'herbal high' preparations, which are distributed under various names such as *Krypton, K2, or Spice* (Dresen et al., 2010; Arndt et al., 2011). While the main psychoactive components of these preparations are believed to be synthetic cannabinoids and herbs only being used as carriers (Cornara et al., 2013), a recent report identified a series of *K2* products that did not contain any known cannabinoid, but did contain mitragynine as psychoactive compound (Logan et al., 2012). Also purified mitragynine becomes increasingly available on a worldwide scale via the Internet (Boyer et al., 2008; Hillebrand et al., 2010; Schmidt et al., 2011). Emerging reports of Kratom

* Corresponding author at: Section of Addiction Medicine, Department of Psychiatry and Psychotherapy, University Clinic, Friedrich-Alexander-University of Erlangen-Nuremberg, Schwabachanlage 6, Erlangen 91054, Germany.
Tel.: +49 09131 85 36896; fax: +49 09131 85 36002.
E-mail address: Christian.Mueller@uk-erlangen.de (C.P. Müller).

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use in the US and Europe suggest a considerable abuse potential with adverse health effects (Boyer et al., 2007, 2008; Havemann-Reinecke, 2011; Ward et al., 2011). Single case reports from Europe and the US are currently accumulating (Boyer et al., 2007, 2008; McWhirter and Morris, 2010; Havemann-Reinecke, 2011; Sheleg and Collins, 2011) which suggest an abuse and addiction potential, toxicity, and fatal interactions with other psychoactive drugs (Kapp et al., 2011; Neerman et al., 2013). A considerable addiction potential is now also supported by systematic animal studies (Yusoff et al., submitted for publication).

Kratom preparations are widely available on the Internet (Hillebrand et al., 2010; Schmidt et al., 2011). They are offered as resin, dried leaf, or powder under the names 'Kratom', 'Mitragyna', 'Concentrated Kratom', 'Plant sample Kratom', and many more. However, qualities vary considerably (Hanna, 2012). The long history of Kratom use in Southeast Asia allows western users via the Internet to access balanced and occasionally scientifically confirmed information on safety issues, dose patterns, potential side effects, and addiction potential of the drug (Siebert, 2012). In Malaysia, Thailand, and many EU countries, mitragynine and Kratom preparations are controlled drugs. In the US, UK, and Germany, they are currently not controlled substances but under surveillance awaiting more scientific evidence (EMCDDA, 2012; DEA, 2013; Hassan et al., 2013). Here we present the first systematic study of Kratom dependence, withdrawal symptoms, and craving in a human population of regular Kratom users in Malaysia.

2. Method

2.1. Study design

A cross-sectional study was conducted between January and December 2012 in three northern states (Penang, Perlis, and Kedah) of the peninsular Malaysia which neighbour the border of south Thailand. These are the states where Kratom is most prevalent in Malaysia. A total of 293 male Kratom users from various community settings were recruited through purposive sampling. Staff from the National Anti-Drug Agency and village heads helped introducing key informants who were familiar with the Kratom users in the respective communities. Key informants were briefed on the objective of the study and also trained how to approach Kratom users in the community. The tests were carried out in community settings including coffee-shops, community halls, clandestine Kratom makeshifts, and respondent's house using structured questionnaires which were translated into the local Malay language. Each interview session lasted between 30 and 45 min. Respondent were compensated with RM 20 (approx. USD 6) for their willingness to participate in the survey.

Urine toxicology tests for methamphetamine, amphetamine, opiates, cannabis, methadone, benzodiazepine, buprenorphine, and ketamine were conducted to exclude respondents who were tested positive for these drugs. This was to ensure that the reported behavioural effects were based on Kratom use only, and not due to the effects of other drugs. Importantly, respondents who used Kratom for less than six months were excluded from the study as they did not yet represent a regular use. The study was approved by the Human Ethics Committee of the Universiti Sains Malaysia.

2.2. Tests and data analysis

At present, there is no tool specifically designed to measure Kratom dependence, withdrawal symptoms, and craving in humans. Thus the Leeds Dependence Questionnaire (LDQ; Raistrick et al., 1994), Marijuana Withdrawal Checklist (MWC; Budney et al., 1999), and Marijuana Craving Questionnaire-Short Form (MCQ-SF; Heishman et al., 2009) were used in this study. We furthermore determined whether duration of use has an effect on severity of dependence, withdrawal, and craving in regular Kratom users. Respondents who had less than three years history of Kratom use were grouped as *medium-term user*, while those with more than three years history were grouped as *long-term user* and responses were compared between both groups.

2.3. Mitragynine analysis

In order to assess the amount of the psychoactive active compound in the local preparations, we measured mitragynine content in three Kratom juice samples as they are typically consumed. One fresh Kratom juice preparation (350 ml) was obtained at each of the three test sites. Chromatographic analysis was performed on an Agilent 1100 liquid chromatography system equipped with manual injector, quaternary HPLC pump, UV detector and ChemStation software for data collection and analysis. The separation was performed on a Gemini-NX 5u C18 reversed phase column (Phenomenex, 10 mm × 4.6 mm) with a 20 μl injection volume. The mobile phased consisted of ammonium acetate, pH 5 (A) and acetonitrile (B). The flow rate was 1 ml/min with isocratic flow of A:B 65:35.

2.4. Statistical analysis

Respondents in this study were divided into two groups based on their Kratom use history. Those who had a less than three year history of Kratom use were grouped as *medium-term user* while those with a more than three year history of Kratom use were grouped as *long-term user*. The respective odds ratios (OR) and 95% confidence intervals (CI) were analysed for both groups. Chi-square tests were used to analyse the differences in Kratom dependence, withdrawal, and craving severity between medium- and long-term Kratom users. All statistical analyses were performed using SPSS 20 and VassarStats (http://vassarstats.net/). A significance level of $p < 0.05$ was used.

3. Results

3.1. Sociodemographic characteristics of Kratom users

Respondents in this study were all male Kratom users ($n = 293$), with the majority of Malay ethnicity. Respondents mean age in this study was 28.9 years. Nearly half were 18–25 years old. Less than 58% (171/293) were single. Two thirds have completed upper-secondary education (193/293) and more than two thirds held an employment (248/293). The majority worked as fisherman, farmers, drivers, and manual labourers. More than half earned between RM 100 and 1000 a month (the average monthly income in Malaysia is RM 5000; The *Star*, 31 March 2013). About 36% of the respondents were ex-drug users (107/293). More than half of the respondents (155/293) had 1–3 years history of Kratom use. Only 10% (28/293) had a ≥10 year history of Kratom use. Respondents mean age of first Kratom use in this study was 24.7 years. Almost half of them started using Kratom between the ages of 11–21 years. The average frequency of current Kratom consumption in the respondents was 3.5 times per day with a quantity of one glass Kratom juice per consumption episode. Each glass contains approx. 350 ml of fresh Kratom juice. About 13% (39/293) consumed 0.5–1.5 glasses of Kratom per day, 42% (124/293) consumed 2–3 glasses per day, and 44% (130/293) more than 3 glasses. Long-term user differed significantly from medium-term users in virtually all sociodemographic parameters (Table 1).

3.2. Kratom preparation and use

The leaves of the Kratom plant were used to prepare Kratom juice. One commonly reported way is to boil the leaves together with clean tap-water for approx. 3–4 h, until the mixture begins to emit a 'strong smell'. Consumers prefer to drink fresh Kratom juice which can be either served warm or chilled. Kratom juice is usually sold in packed plastic bags each containing about 250–350 ml. Although it is an offence to sell Kratom in Malaysia, it is sold furtively in few selected food-stalls frequented predominantly by manual labourers. Kratom is directly consumed from the purchased pack. It is commonly poured into a glass and drunk slowly during leisure time. Beginners prefer to mix sweet beverages (e.g., coke) with their Kratom drink in order to mask its bitter taste. Those who wish to obtain a better 'euphoria' or 'kick' usually mix cough-syrup (Dextromethorphan) or Erimin 5 (Nimetazepam) with the Kratom drink. The cost for processed Kratom juice varies and geographic factors influence the price. A pack Kratom (approx. 250 ml) was sold at the time of the testing for a price of RM 2.50–5.00. Regular Kratom users may consume a minimum of 3–4 packs per day. Usually, Kratom users do not know the purity level of their processed juice.

Table 1
Difference between medium- and long-term Kratom users ($n = 293$).

			Medium-term user		Long-term user		p-Value
	n=	%	$n = 153$	%	$n = 140$	%	
Marital status							
Married	118	40	52	34.0	66	47.1	**0.022***
Single/divorced	175	60	101	66.0	74	52.9	
Employment status							
Employed	248	85	122	79.7	126	90.0	**0.015***
Unemployed	45	15	31	20.3	14	10.0	
Accommodation							
Parents	142	48.5	92	60.1	50	35.7	**0.001***
Family/friends/others	151	51.5	61	39.9	90	64.3	
Income							
<1000	192	65.5	111	72.5	81	57.9	**0.008***
≥1001	101	34.5	42	27.5	59	42.1	
Age							
18–25 years	132	45	84	54.9	48	34.3	0.001*
>26 years	161	55	69	45.1	92	65.7	
Ex-addict							
Yes	107	36.5	46	30.1	61	43.6	**0.016***
No	186	63.5	107	69.9	79	56.4	
Family use							
Yes	80	27	34	22.2	46	32.9	**0.041***
No	213	73	119	77.8	94	67.1	
Need to use ketum daily							
Yes	230	78.5	113	73.9	117	83.6	**0.043***
No	63	21.5	40	26.1	23	16.4	

* *Note*: Bold denote p-value (<0.05).

3.3. Reason for using Kratom

Respondents in this study reported to use Kratom for many different reasons. The majority used Kratom to enhance their physical energy. More than one third used Kratom because of curiosity and peer influence. About 15% (45/293) used Kratom to abstain from illicit drugs and alcohol, while about 13% (38/293) used Kratom to treat their medical problems (e.g., diabetes). Others used Kratom to improve their mood and overcome fatigue.

3.4. Kratom dependence

All regular users in this study claimed to be dependent on Kratom. More than half of the respondents (161/293) faced severe Kratom dependence problems, while 45% (132/293) had moderate Kratom dependence problems. More than 89% (262/293) of the regular users tried to abstain from Kratom in the past. About 90% (265/293) claimed that they have better social functioning when using Kratom. This was mainly because they were able to work long hours and to better socialise with their family members and friends. Almost 79% (230/293) reported that they needed to use Kratom daily. About 32% (95/293) of the respondents had increased their Kratom intake, while 42% (124/293) maintained their intake at rather constant level since the onset of their Kratom use.

Those who used more than 3 glasses of Kratom daily were more likely to report severe Kratom dependence than those who consumed less than 3 glasses (OR: 7.05; 95% CI; 4.09–12.13; $p < 0.001$). Similarly, those who used Kratom ≥3 times daily, were 5.19 times more likely to report severe Kratom dependence (OR: 5.19; 95% CI; 3.02–8.92; $p > 0.001$).

3.5. Kratom withdrawal – physiological symptoms

Physiological withdrawal symptoms encountered by Kratom users during withdrawal include sleeping difficulty, decreased appetite, nausea, vomiting, muscle spasm, sweating, fever, abdominal pain, diarrhoea, headaches, hot flashes, watery eyes and nose, hiccups, and shakiness or tremors. About 76% (222/293) of the Kratom users experienced body aches, including severe muscle pain and cramps, after abstaining abruptly from Kratom use. About 65% (190/293) experienced mild withdrawal effects while 35% (103/293) experienced moderate to severe withdrawal effects after Kratom cessation. Kratom withdrawal symptoms were experienced for 1–3 days in 64% (187/293) of the regular users. In 36% (106/293) they lasted for more than 3 days.

Those who consumed ≥3 glasses of Kratom daily were more likely to report severe withdrawal symptoms during Kratom cessation (OR: 4.63; 95% CI; 2.46–8.71; $p < 0.001$), compared to those who drank less than 3 glasses of Kratom daily.

3.6. Kratom withdrawal – psychological symptoms

Psychological withdrawal symptoms commonly reported by the respondents in this study include nervousness, sadness, restlessness, anger, tension, and depressed mood. About 73% (151/293) of the respondents encountered at least five different psychological withdrawal symptoms during the first day of Kratom cessation. None of the respondents had any suicidal ideation after using Kratom for prolonged periods.

3.7. Kratom craving

About 23% (68/293) of the regular users reported high craving for Kratom while 77% admitted low craving. Those who consumed ≥3 glasses of Kratom daily were more likely to report higher craving for Kratom than those who consumed less than 3 glasses (OR: 4.8; CI: 2.09–11.10: $p < 0.001$). Only 2% (6/293) of the respondents have sought treatment for their Kratom use problems.

3.8. Medium-term vs. long-term Kratom use

There were no statistically significant differences in the reasons for Kratom use between medium- and long-term users ($p > 0.05$; Table 2). There were also no significant differences in Kratom dependence severity, withdrawal severity, and craving between medium-term and long-term Kratom users in this study ($p > 0.05$).

Table 2
Reasons for using Kratom.

			Medium-term user		Long-term user	
	n=	%	n=153	%	n=140	%
To enhance physical energy	83	28	44	28.8	39	27.9
Curiosity	61	21	35	22.9	26	18.6
Peer influence	46	16	30	19.6	16	11.4
To abstain from illicit drugs/alcohol	45	15	15	9.8	30	21.4
Self-treatment	38	13	22	14	16	11.4
To improve mood/ease boredom	17	6	6	3.9	11	7.9
To relieve fatigue	3	1	1	1	2	1.4

3.9. Mitragynine analysis

A consumption episode usually comprises the ingestion of one glass of Kratom juice (approx. 350 ml). Results from the analysis revealed a mitragynine content in the 350 ml Kratom juice sample acquired from Perlis of 83.4 mg, from Penang of 78.9 mg, and from Kedah of 74.6 mg (mean: 79.0 mg). On average, respondents in this study used 3.5 glasses of Kratom daily in each region. This means respondents ingested daily an average amount of 261.1–291.9 mg (mean: 276.5 mg) of mitragynine.

4. Discussion

Kratom is a widely used psychoactive drug in Southeast Asia which is currently spreading to other parts of the world (e.g., Forrester, 2013). Although anecdotal reports are available (Suwanlert, 1975), this study is the first to measure systematically Kratom dependence, withdrawal symptoms, and craving in regular Kratom users in the northern states of the peninsular Malaysia. All the respondents in this study were dependent on Kratom and also encountered unpleasant withdrawal symptoms and craving after trying to abstain from Kratom use.

Kratom is viewed as a cheap psychotropic drug with dual-properties. It can be used as a stimulant drug to enhance physical tolerance, or as an analgesic due to its pain-relieving properties. All respondents in this study were dependent on Kratom. They believed that Kratom is not as harmful as other available drugs, such as methamphetamine or heroin. Most users shared the belief that it is better to use Kratom in order to improve work performance than using illicit stimulant-drugs which would also be more expensive. Those who claimed that they use Kratom to enhance their work performances, actually use Kratom as an 'energy boosting drink'. Respondents claimed that Kratom induces stimulant like-effects when it is use in small quantity especially under the burning sun or when engaging in an exhausting work. Sedating effects, in turn, are reported when Kratom is used excessively or when slowly consumed at leisure time, e.g., at social gatherings with friends. The Malay village community does usually not discriminate Kratom users because they regard the Kratom using practice as an inherent aspect of their ancestral tradition. They see the Kratom using habit as an integral part of their present culture, and, hence, Kratom users as 'diligent' and 'hard working', while e.g., cannabis users are 'lazy' (Saingam et al., 2013). This is in line with anecdotal reports were Kratom users became dependent to Kratom because they wanted to work more efficiently in their rice mills (Suwanlert, 1975). There was a very low rate of treatment seeking among the regular Kratom users in this study. They viewed their Kratom dependence as non-problematic and something which they could deal with by themselves. At this point it will be interesting to assess behavioural impairments that result from regular Kratom use and dependence. Systematic research on this question is warranted.

In Southeast Asia, Kratom use may represent another case of systematic drug instrumentalization on daily basis (Müller and Schumann, 2011a). Psychoactive drugs can be instrumentalized for various purposes. Thereby, the drug-induced change in the mental state facilitates the more effective pursuit of another, originally drug-independent task. It was claimed that most of the non-addicted psychoactive drug use in the world is based on drug instrumentalization rather than on the direct pharmacological reinforcing effects of the drug (Müller and Schumann, 2011a). However, a successful instrumentalization may under certain circumstance pave the way to enhanced and prolonged drug use, which may eventually lead to drug addiction (Müller and Schumann, 2011b). From the reports of Kratom use in order to enhance work output and to recover from exhausting work, one may speculate that the easy availability of this 'drug instrument' and the lack of alternative instruments in rural Malaysia may foster systematic Kratom use and the transition to Kratom dependence.

Findings from this study showed that more than half of the respondents had severe Kratom dependence problems. All others had moderate Kratom dependence problems. A sizable proportion of the respondents in this study could usually not endure the first day of Kratom withdrawal symptoms as the symptoms disrupt their physical and psychological functioning. Although the withdrawal symptoms lasted only for a short period of a few days and gradually subsided, the pain that they experienced severely affected their work performance and mental occupation. Most respondents tried to self-medicate their withdrawal symptoms with sleeping pills. The withdrawal symptoms in this study were similar to the symptoms reported in previous reports from Malaysia and Thailand (Suwanlert, 1975; Assanangkornchai et al., 2007a,b; Vicknasingam et al., 2010; Ahmad and Aziz, 2012; Saingam et al., 2013), but are less severe than those reported after excessive Kratom consumption in the US or Europe (McWhirter and Morris, 2010; Sheleg and Collins, 2011; Kapp et al., 2011). The reason for this discrepancy may be a better social control of the consumption in regions with historically established Kratom consumption and a somewhat better predictability of the Kratom quality. Also, the use of highly concentrated plant extracts as it was reported in Europe and the US may lead to a significant escalation and stronger withdrawal effects in Kratom consumers (Havemann-Reinecke, 2011).

In this study, those who drank Kratom several times per day for more than 6 month were regarded as regular Kratom users. Thirty-six percent of the respondents had previous history of substance abuse problems. When probed, most respondents reported being previously dependent on cannabis, heroin, and methamphetamine. Respondents claimed that they use Kratom juice to treat and reduce their dependence on illicit drugs. In fact now they used Kratom as a substitute for the illicit drugs. None of the respondents were tested positive for using illicit drugs during the interview which may suggest a certain efficacy as a substitute drug. The use of Kratom and mitragynine as a substituting drug for heroin was also reported in the US, although with mixed success (Boyer et al., 2007, 2008; Neerman et al., 2013). To what extent Kratom may be used to substitute heroin and being used effectively for drug addiction treatment is an open question that awaits systematic research.

The average mitragynine content in each glass Kratom was 79.0 mg suggesting a total daily dose of 276.5 mg mitragynine in this study. This is more than previously estimated in Thailand and Malaysia (Suwanlert, 1975; Vicknasingam et al., 2010; Saingam et al., 2013). Amounts of mitragynine consumed by western Kratom abusers are currently unclear. On average, regular Kratom users in this study used Kratom 3.5 times per day. Most of them used Kratom in the morning, afternoon, and evening. Kratom users reported that they need to use Kratom in the morning to boost their energy levels and to motivate themselves to work. In the evenings, they normally used Kratom to combat fatigue and to help them to 'mingle and chat' with their colleagues in the village.

Craving for Kratom was reported by all regular users with most of them admitting a moderate level. High craving coincided with high Kratom consumption, but was not dependent on the length of the consumption. This is in line with case reports from western users, who showed a strong craving and subsequent escalation of consumption (Havemann-Reinecke, 2011). At present, there is no specific treatment for Kratom dependence. There have been cases where Kratom users try to use methadone and benzodiazepines to treat their Kratom dependence. The unsupervised use of methadone and benzodiazepines among Kratom users could impose an additional risk of overdose (Neerman et al., 2013). Many habitual Kratom users are unable to quit from Kratom use, because of its chronic withdrawal symptoms, e.g., sleeping problems and chronic pain during the first day of abrupt cessation.

This study is the first to systematically measure Kratom dependence, withdrawal, and craving in regular Kratom users. It clearly shows in a population of Malaysian consumers that regular Kratom use may lead to drug dependence with profound withdrawal symptoms and subsequent drug craving in circumstances where consumption is socially controlled. These findings may also suggest a closer monitoring of Kratom preparations and of 'legal high' preparations which contain Kratom in regions where the availability and consumption only recently emerged (Logan et al., 2012; Forrester, 2013). Findings from this study may help medical practitioners to better understand the effects of prolonged Kratom use in humans. Its efficacy as an adjunct for opiate addiction therapy, however, might need thorough investigation from a broader perspective.

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The funders had no role in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

Contributors

DS and BKV designed the study. DS, BKV, and CPM obtained the funding. DS performed the experiment. DS, BKV, and CPM analysed the data and wrote the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors reported no biomedical financial interests or potential conflicts of interest.

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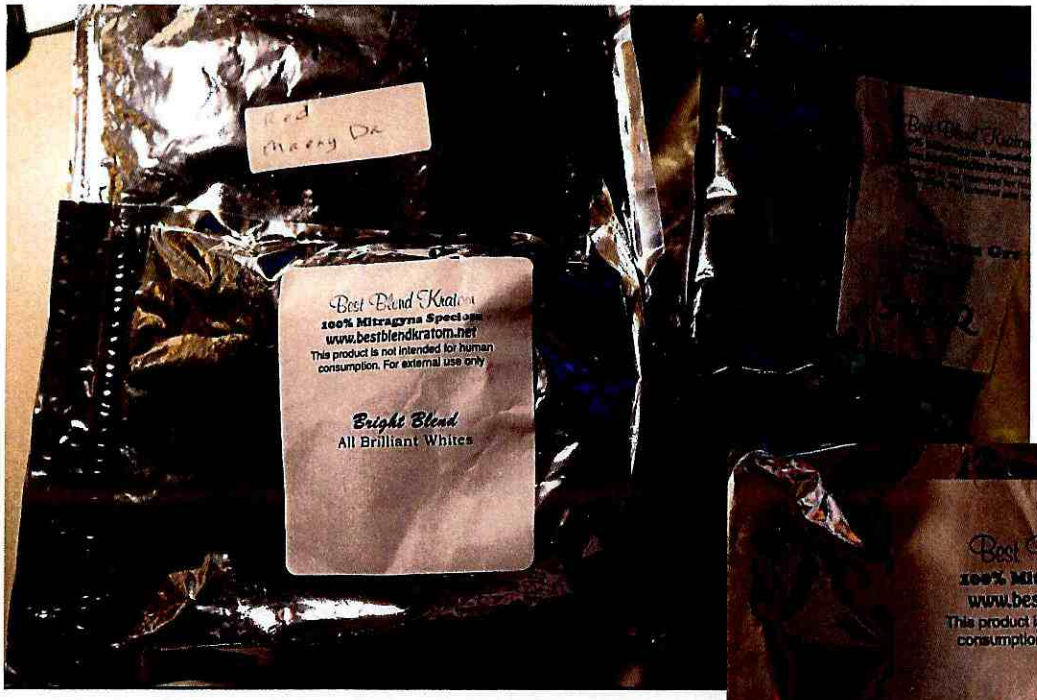
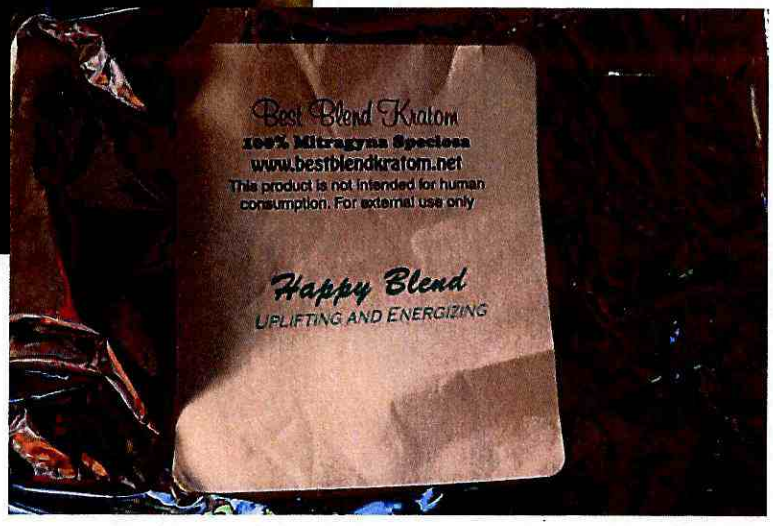
References

Adkins, J.E., Boyer, E.W., McCurdy, C.R., 2011. *Mitragyna speciosa*, a psychoactive tree from Southeast Asia with opioid activity. Curr. Top. Med. Chem. 11, 1165–1175.
Ahmad, K., Aziz, Z., 2012. *Mitragyna speciosa* use in the northern states of Malaysia: a cross-sectional study. J. Ethnopharm. 141, 446–450.
Arndt, T., Claussen, U., Gussregen, B., Schrofel, S., Sturzer, B., Werle, A., Wolf, G., 2011. Kratom alkaloids and O-desmethyltramadol in urine of a krypton herbal mixture consumer. Forensic Sci. Int. 208, 47–52.
Assanangkornchai, S., Pattanasattayawong, U., Samangsri, N., Mukthong, A., 2007a. Substance use among high-school students in Southern Thailand: trends over 3 years (2002–2004). Drug Alcohol Depend. 86, 167–174.
Assanangkornchai, S., Muekthong, A., Samangsri, N., Pattanasattayawong, U., 2007b. The use of *Mitragynine speciosa* (krathom), an addictive plant in Thailand. Subst. Use Misuse 42, 2145–2157.
Babu, K.M., McCurdy, C.R., Boyer, E.W., 2008. Opioid receptors and legal highs: salvia divinorum and kratom. Clin. Toxicol. (Phila.) 46, 146–152.
Boyer, E.W., Babu, K.M., Macalino, G.E., Compton, W., 2007. Self-treatment of opioid withdrawal with dietary supplement, kratom. Am. J. Addict. 16, 352–356.
Boyer, E.W., Babu, K.M., Adkins, J.E., McCurdy, C.R., Halpern, J.H., 2008. Self-treatment of opioid withdrawal using kratom (*Mitragyna speciosa* korth.). Addiction 103, 1048–1050.
Budney, A.J., Novy, P.L., Hughes, J.R., 1999. Marijuana withdrawal among adults seeking treatment for marijuana dependence. Addiction 94, 1311–1322.
Cornara, L., Borghesi, B., Canali, C., Andrenacci, M., Basso, M., Federici, S., Labra, M., 2013. Smart drugs: green shuttle or real drug? Int. J. Legal Med. 127, 1109–1123.
Dresen, S., Ferreirós, M., Pütz, M., Westphal, F., Zimmermann, R., Auwärter, V., 2010. Monitoring of herbal mixtures potentially containing synthetic cannabinoids as psychoactive compounds. J. Mass Spectrom. 45, 1186–1194.
Drug Enforcement Administration (DEA), 2013. Kratom (*Mitragyna speciosa* korth). http://www.deadiversion.usdoj.gov/drug_chem_info/kratom.pdf (accessed on 10.11.13).
EMCDDA, 2012. http://www.emcdda.europa.eu/publications/drug-profiles/kratom/de (accessed on 10.06.12).
Forrester, M.B., 2013. Kratom exposures reported to Texas poison centers. J. Addict. Dis. 32, 396–400.
Hanna, J., 2012. Bogus Kratom market exposed. Entheogen. Rev. 12, 26–28.
Hassan, Z., Muzaimi, M., Navaratnam, V., Yusoff, N.H., Suhaimi, F.W., Vadivelu, R., Vicknasingam, B.K., Amato, D., von Hörsten, S., Ismail, N.I., Jayabalan, N., Hazim, A.I., Mansor, S.M., Müller, C.P., 2013. From kratom to mitragynine and its derivatives: physiological and behavioural effects related to use, abuse, and addiction. Neurosci. Biobehav. Rev. 37, 138–151.
Havemann-Reinecke, U., 2011. Kratom and alcohol dependence: clinical symptoms, withdrawal treatment and pharmacological mechanisms – a case report. Eur. Psychiatry 26 (Suppl. 1), 01–50.
Heishman, S.J., Evans, R.J., Singleton, E.G., Levin, K.H., Copersino, M.L., Gorelick, D.A., 2009. Reliability and validity of a short form of the Marijuana Craving Questionnaire. Drug Alcohol Depend. 102, 35–40.
Hillebrand, J., Olszewski, D., Sedefov, R., 2010. Legal highs on the Internet. Subst. Use Misuse 45, 330–340.
Kapp, F.G., Maurer, H.H., Auwärter, V., Winkelmann, M., Hermanns-Clausen, M., 2011. Intrahepatic cholestasis following abuse of powdered kratom (*Mitragyna speciosa*). J. Med. Toxicol. 7, 227–231.
Logan, B.K., Reinhold, L.E., Xu, A., Diamond, F.X., 2012. Identification of synthetic cannabinoids in herbal incense blends in the United States. J. Forensic Sci. 57, 1168–1180.
Matsumoto, K., Horie, S., Takayama, H., Ishikawa, H., Aimi, N., Ponglux, D., Murayama, T., Watanabe, K., 2005. Antinociception, tolerance and withdrawal symptoms induced by 7-hydroxymitragynine, an alkaloid from the Thai medicinal herb *Mitragyna speciosa*. Life Sci. 78, 2–7.
McWhirter, L., Morris, S., 2010. A case report if inpatient detoxification after kratom (*Mitragyna speciosa*) dependence. Eur. Addict. Res. 16, 229–231.
Müller, C.P., Schumann, G., 2011a. Drugs as instruments: a new framework for non-addictive psychoactive drug use. Behav. Brain Sci. 34, 293–310.
Müller, C.P., Schumann, G., 2011b. To use or not to use: expanding the view on non-addictive psychoactive drug consumption and its implications. Behav. Brain Sci. 34, 328–347.
Neerman, M.F., Frost, R.E., Deking, J., 2013. A drug fatality involving kratom. J. Forensic Sci. 58 (Suppl. 1), S278–S279.
Raistrick, D., Bradshaw, J., Tober, G., Weiner, J., Allison, J., Healey, C., 1994. Development of the Leeds Dependence Questionnaire (LDQ): a questionnaire to measure alcohol and opiate dependence in the context of a treatment evaluation package. Addiction 89, 563–572.
Saingam, D., Assanangkornchai, S., Geater, A.F., Balthip, Q., 2013. Pattern and consequences of krathom (*Mitragyna speciosa* korth.) use among male villagers in southern Thailand: a qualitative study. Int. J. Drug Policy 24, 351–358.
Schmidt, M.M., Sharma, A., Schifano, F., Feinmann, C., 2011. Legal highs on the net-Evaluation of UK-based Websites, products and product information. Forensic Sci. Int. 206, 92–97.

Sheleg, S.V., Collins, G.B., 2011. A coincidence of addiction to Kratom and severe primary hypothyroidism. J. Addict. Med. 5 (4), 300–301.
Siebert, D., 2012. The Kratom User's Guide. www.sagewisdom.org/kratomguide.html (accessed on 14.02.14).
Suwanlert, S., 1975. A study of kratom eaters in Thailand. Bull. Narcot. 27, 21–27.
Vicknasingam, B., Narayanan, S., Beng, G.T., Mansor, S.M., 2010. The informal use of ketum (*Mitragyna speciosa*) for opioid withdrawal in the northern states of peninsular Malaysia and implications for drug substitution therapy. Int. J. Drug Policy 21, 283–288.
Ward, J., Rosenbaum, C., Hernon, C., McCurdy, C.R., Boyer, E.W., 2011. Herbal medicines for the management of opioid addiction: safe and effective alternatives to conventional pharmacotherapy? CNS Drugs 25, 999–1007.
Yusoff, N.H.M., Suhaimi, F.W., Vadivelu, R.K., Hassan, Z., Rümler, A., Rotter, A., Amato, D., Dringenberg, H., Mansor, S.M., Navaratnam, V., Müller, C.P., 2013 Abuse potential and adverse cognitive effects of mitragynine (kratom) in rats. submitted for publication.





Photographs of Kratom products located in the residence of an accidental overdose victim on March 4, 2018. (NE Kansas)