Kratom (*Mitragyna Speciosa*) as a Potential Therapy for Opioid Dependence

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Several psychoactive herbal products are widely available over the Internet with minimal control on their sale. Complicating this availability is the poor understanding of the chemical components and pharmacology of such products. Very little is known about specific chemical entities or combinations of chemicals present in these products. The availability of these products to adolescents and young adults has created a great concern for understanding the chemistry, psychopharmacology, and toxicology of these herbs. Use and abuse of these substances is difficult to measure, other than through anecdotal reports found on websites and through media reports. Particular interest has been generated around kratom (*Mitragyna speciosa* [Korth] Havil.), as it has been on the DEA (United States Drug Enforcement Agency) List of Drugs and Chemicals of Concern for over a decade (DEA, 2016). Kratom has been touted as a "legal high", and the major alkaloid, mitragynine, has been thought to be responsible for its actions at opioid receptors (Babu et al., 2008). In addition, a minor alkaloid and oxidative product of mitragynine, 7-hydroxymitragynine, has also been reported to have potent agonist activity at opioid receptors (Babu et al., 2008). Although 7-hydroxymitragynine occurs in trace amounts in the natural plant, several marketed products are suspected to be adulterated with increased levels of this compound (Lydecker et al., 2016). According to the scientific literature, it is not clear if mitragynine has abuse liability, and has been reported to have mild analgesic properties most similar to codeine or non-steroidal anti-inflammatory drugs (NSAIDs) (Macko et al, 1972). Conversely, 7-hydroxymitragynine (a minor plant constituent), when purified and pharmacologically tested alone, does show a conditioned place preference (drug-seeking behavior) in rodents, as well as potent analgesia (Matsumoto et al., 2008). To complicate matters, it is not entirely known if 7-hydroxymitragynine is produced by the plant, or is an oxidative byproduct of leaf drying, due to the low amounts in which it has been reported to occur in traditional fresh leaf extracts. Synthetic procedures have been published to convert mitragynine to 7-hydroxymitragynine (Takayama et al., 2002), but this involves specialty chemicals that are not commonly available to the public or clandestine laboratories. Nonetheless, from those commercially available products analyzed, it is clear that the levels of 7-hydroxymitragynine are in much greater concentrations than occur in nature (Lydecker et al, 2016).

Kratom has been linked to 16 deaths, although in each case the deceased individuals had multiple substances in their systems. It is important to note that not a single death has been attributed to kratom in Southeast Asia, where it has been traditionally used for over 100 years. In addition, the DEA had rightfully banned synthetic bath salts and synthetic cannabinoids (i.e., K2 or Spice) based on scientific evidence, removing them from the consumer marketplace and providing them a home in the list of Schedule I controlled substances. This void in the consumer market was filled with kratom products in gas stations, herbal shops, and the Internet. The DEA faced pressure from a small but vocal section of the public to ban kratom and place it in Schedule I. Even though very limited scientific information was available on kratom, in the fall of 2016 the DEA nevertheless announced

their intention to place kratom, mitragynine, and 7-hydroxymitragynine into Schedule I of the Controlled Substances Act (DEA, 2016). This created a large push from those that have utilized kratom for control of pain and prescription opioid addiction to place pressure on the DEA as well. The result was unprecedented, with the DEA announcing a 30-day open comment period for the public. Over 23,000 written pleas were received by the DEA to reconsider this position (Federal register 2016). These communications came from the general public, legislators, and the scientific community involved in kratom research. In addition, the Botanical Education Alliance published an 8-Factor analysis of kratom by Dr. Jack E. Henningfield that indicated kratom is *not* addictive (Pinney Associates, 2016). Moreover, it stated that the factors that appear important in maintaining kratom use appear more similar to those of normal caffeine intake. This report was submitted to the DEA & FDA in 2016. For the first time in history, the DEA withdrew their intention to place Kratom into Schedule I, though it still has the right to do so at any time (Federal Register, 2016). This manuscript aims to provide the current state of science around kratom.

Kratom is a tree native to Thailand, Malaysia, and other areas of Southeast Asia. The leaves of this tree have been utilized for many years by laborers for their stimulant effects (at low doses), and their ability to invigorate workers in harsh conditions (Jansen et al., 1988). Kratom has also seen much use as a replacement for opium due to its euphoric and sedative effects (at higher doses) (Jansen et al., 1988). Extracts and decoctions have also been noted as a method to alleviate opioid withdrawal. (Jansen et al., 1988; Boyer et al.,2007, 2008). Kratom was outlawed in Thailand through the Kratom Act in 1943; however, it remains a widely popular substance there (DEA, 2017; Jansen et al., 1988). It has been assumed that the ban in Thailand was due to the government's inability to generate tax revenue from the plant, although this is not documented. With the reports of its actions, and the fact that it is not controlled in much of the world, it was introduced to the Western world via the Internet and touted for its stimulant and opium-like effects. Indeed, according to an Internet supplier, sales are very good in the United States[1].

Currently, kratom is not a controlled substance under federal law in the United States, and little information is known on its true pharmacological activities. However, six states have banned kratom as of July 2017: Alabama, Arkansas, Indiana, Tennessee, Vermont, and Wisconsin. It has also been made illegal in Sarasota County, Florida; San Diego, California; and Jerseyville, Illinois. In addition, it is illegal in many countries, including Australia, Burma, Denmark, Lithuania, Malaysia, Myanmar, Poland, Sweden, Thailand, and Vietnam (Kratom Science, 2017).

Extracts of *Mitragyna speciosa* have been used in Thailand and Malaysia for many years for their opium-like effects and coca-like stimulant ability to combat fatigue (Jansen et al., 1988). It is interesting that the plant seems to have these apparently contradictory effects. Some early studies (Wray et al., 1907a; Wray et al., 1907b) indicated a similarity to cocaine in humans, but other studies (Jansen et al., 1988; Takayama 2004; Boyer et al., 2007) have shown opioid-like effects. In fact, kratom has long been promoted in these areas as a substitute for opium, and has also been used to wean addicts off morphine. Some recent clinical reports of kratom being utilized as a self-treatment for opioid withdrawal indicate the medical community is seeing patients who are using kratom (Jansen et al., 1988; Takayama 2004; Babu et al., 2008; DEA Public Affairs, 2016). The plant material and extracts are available on the Internet, making them easily obtainable by those who may want to experiment with such substances. Currently, as mentioned, there are no restrictions on this plant, extracts, or purified compounds in the majority of the United States.

Fortunately, there is some information in the literature about the chemistry and pharmacology of this plant. Some active alkaloids on opioid receptors have been identified from extracts of *Mitragyna speciosa* (Takayama et al., 2002) and are shown in Figure 1. These include: mitragynine (1), 7-hydroxymitragynine (2), and corynantheidine (3).

The studies that have been reported focus on the opioid-like effects of extracts and some of the pure chemicals that have been isolated. Many studies on these substances demonstrate effects that are reversible with naloxone, an opioid-receptor antagonist. It has been reported that extracts of *Mitragyna speciosa* can reduce pain in animal studies. Interestingly, little is known about the mechanism of the reported stimulant actions. A review of the literature demonstrates a wide variety of opioid activity and inconsistency in studies. These inconsistencies range from extraction procedures, binding affinity measurements, and antagonists utilized, as well as *in vivo* reports. These are all detailed below, and underscore the strong need to understand the actions of the extract and isolated natural products in a side-by-side comparison in the same assays to more completely understand the chemistry and pharmacology of this species.

1 Salesperson at Naturalorganix.com. Personal communication 8/30/2017

Isolation of some 40 alkaloids from this plant have focused on the most predominant alkaloid, mitragynine (1) (Adkins et al., 2011). Mitragynine, a corynantheidine alkaloid first isolated by Field in 1921 (Field 1921), has demonstrated opioid-receptor affinity and partial agonist activity. Interestingly, opioid-receptor affinities that have been reported in multiple studies are not consistent. In some cases, affinities for some opioid receptor subtypes have been reported by some and not found by others. This may be due to variations in the purity of compounds, receptor preparations, and radioligands utilized in these studies. However, it does lead to an ambiguity of the understanding of these naturally occurring chemical components. Of high interest are the most recent reports that demonstrate mitragynine as a partial agonist that has a G-protein signaling bias (Kruegel et al., 2016). This signaling bias has been hypothesized to result in fewer liabilities from mitragynine than other opioid ligands. Most notably, mitragynine and kratom do not cause significant respiratory depression in rodents, nor presumably in humans. This pharmacology may explain why there have not been any reports of overdose deaths from kratom alone, as unlike traditional morphine-based opioids, there seems to be little to no effect on respiration. If kratom has been listed as a cause of death, it is still suspect, as there are no controls on what products are sold. This lack of control and standardization makes the marketplace a "buyer beware" one. Moreover, it can be almost impossible to analyze a product for an adulterant that is not known. This has been evidenced by the rapidly changing landscape of compounds that are found in synthetic cannabinoid or bath salt preparations.

The first reports of pharmacological studies on mitragynine appeared in the literature in 1972 (Macko et al., 1972). Researchers at Smith, Kline and French (SKF) were interested in finding a novel analgesic that would have less liability than the currently utilized opioids (i.e. morphine). Their studies were the most comprehensive at the time and still remain as one of the more complete in the literature. A battery of animal studies were undertaken to investigate the analgesic potential and opioid actions of mitragynine. These studies did show that mitragynine had analgesic and antitussive properties comparable to codeine. Unlike codeine, mitragynine did not produce emesis or dyspnea, was not blocked by nalorphine, and had much less respiratory depression. Interestingly, it could suppress the opioid withdrawal syndrome. Moreover, it was noted that mitragynine was active only via the oral and intraperitoneal routes of administration (in an equal ratio), and was inactive via the subcutaneous route. It was hypothesized that the analgesic activity may be related to a metabolite, or that the bioavailability of mitragynine is influenced by the acidic conditions of the route of administration. It appeared that SKF decided to abandon further studies on this substance, most likely due to the weak analgesic potency when compared to traditional, marketed opioid pharmaceuticals.

Mitragynine seemed to be discounted for a number of years until the mid-1990s, when researchers in Japan began to study this compound and plant species again. By this time, it had been realized that nalorphine had mixed opioid agonist/antagonist actions, and may have confounded the results previously reported in the study carried out at SKF. The analgesic activity of mitragynine was again investigated in the tail-pinch and hot-plate tests, resulting in antinociceptive activity that was completely abolished by naloxone, a pure opioid receptor antagonist (Matsumoto et al., 1996a). This indicated the involvement of supraspinal opioid receptors in the analgesic actions of mitragynine and sparked a renewed interest in the pharmacology of this molecule.

Shortly thereafter, the same group indicated the contribution of descending noradrenergic and serotonergic systems in the analgesic activities of mitragynine (Matsumoto et al., 1996b). This is similar to what is known with the actions of morphine. Utilizing the same paradigm in their previous study, the involvement of these systems was investigated by employing the a2-adrenoceptor antagonist idazoxan, and the 5-HT receptor antagonist cyproheptadine. Each of these agents significantly antagonized the analgesic effects of mitragynine. This work indicated that mitragynine may stimulate the release of endogenous norepinephrine and serotonin, similar to the actions of other opioid ligands.

Another study to elucidate the mechanism of action of mitragynine involved the 5-methoxy-N, N-dimethyltryptamine-induced head-twitch response in mice (Matsumoto et al., 1997). This study, again from the same researchers in Japan, seemed to echo the findings of the involvement of noradrenergic and serotonergic mechanisms in the actions of mitragynine. Indeed, mitragynine suppressed the effects of head-twitch in this assay, indicating possible agonist actions on adrenergic and antagonist actions on serotonergic systems.

The inhibition of electrically stimulated contraction in the guinea-pig ileum was also demonstrated to work through opioid mechanisms, as it was reversed by naloxone (Watanabe et al., 1997) This study did not employ any subtype-selective antagonists to indicate the possible opioid-recep-

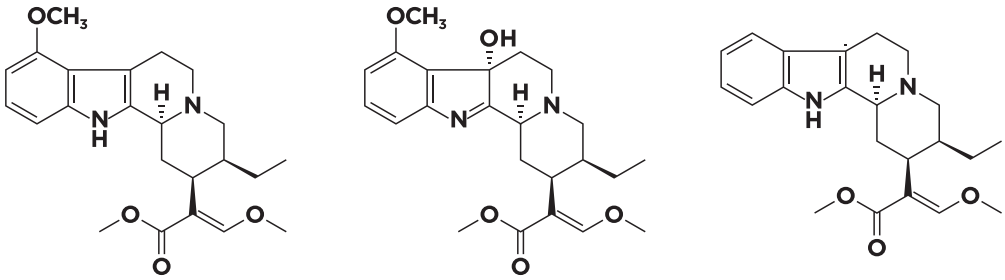

Fig. 1 The opioid-active alkaloids isolated from *Mitragyna speciosa*.

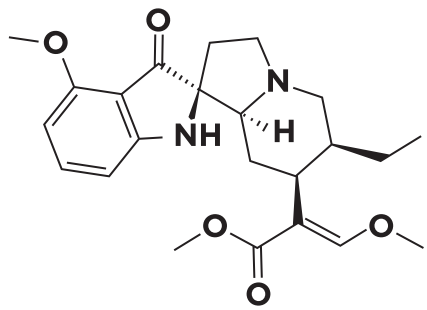

Fig. 2 Structure of the microbial metabolite, mitragynine pseudoindoxyl.

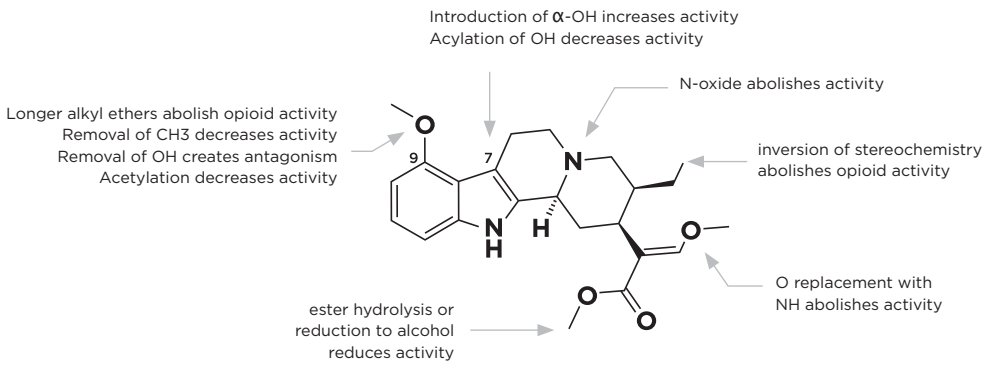

Fig. 3 Known structure-activity relationships of mitragynine.

tor subtypes involved. Some understanding of the subtypes involved was revealed through a study of antinociception in mice, conducted by the same researchers. Their results indicated the involvement of MOP and DOP (Mu and Delta Opioid) receptors through the use of subtype-selective antagonists. They concluded that mitragynine has different selectivity than morphine for opioid-receptor subtypes, yet no receptor binding data was presented. It was much later that the same group published findings on the inhibitory effects of mitragynine on neurogenic contraction of the guinea-pig vas deferens (Matusumoto et al. 2005). The vas deferens is known to contain high amounts of DOP and MOP receptors. In this study, the effects of mitragynine were unable to be blocked by naloxone, leading to the conclusion that opioid receptors are not involved. The conclusion of these studies indicated that the inhibitory effects of mitragynine in this paradigm were through the blockade of calcium channels. This study was confounded by the fact that morphine could not inhibit electrically induced contraction in this assay, leading a reader to question the validity of the findings.

The first receptor-binding data for mitragynine was presented in 2002 (Takayama et al., 2002). The binding affinities for mitragynine at the three opioid receptors were determined using guinea pig brain membranes and reported as pKi values. The data indicated that mitragynine is a MOP-selective opioid ligand with a pKi value of 8.14 ± 0.28, and a relative affinity of 88.7% for the MOP over the DOP and KOP receptors. The pKi values at the DOP and KOP were 7.22 ± 0.21 and 5.96 ± 0.22, respectively. This report did not include functional data at each of the receptors, so it is difficult to relate it to the previously reported receptor selectivity studies that were conducted in mice. As previously mentioned, the *in vivo* study implicated the roles of the MOP and DOP in the actions of mitragynine. Taken together, these data are a bit inconsistent but still reasonable, since functional activities were not presented in the more recent report. The receptor-binding affinities described in this study also included other naturally occurring alkaloids from *Mitragyna speciosa*, and some semi-synthetic derivatives. Most of the natural products and semi-synthetic analogs had much less affinity than mitragynine. Although most of these compounds had less opioid receptor affinity, 7-hydroxymitragynine was 10 times more potent than morphine, and 40 times more potent than mitragynine in the guinea pig ileum assay. Surprisingly, this compound was not investigated in analgesic studies with mice so it was not determined if the activity was due to opioid receptors. The most interesting finding from this work was the inclusion of a previously reported microbial oxidative metabolite of mitragynine, mitragynine pseudoindoxyl (Zarembo et al., 1974; Takayama et al., 2002) (4, Figure 2).

This compound had a higher affinity for mu-opioid receptors (pKi value 10.06 ± 0.39) and a similar relative affinity to morphine among the other opioid receptors (Takayama et al., 2002). Moreover, mitragynine pseudoindoxyl was 35 times more potent than morphine in the electrically stimulated guinea pig ileum assay. However, when mitragynine pseudoindoxyl was tested *in vivo*, the analgesic activity was less than that of morphine, but greater than mitragynine itself. The effects of mitragynine pseudoindoxyl were completely reversed by naloxone. Studies on this molecule have not appeared in the literature since this report. It may be due to the source of this molecule, as it is a metabolite of *Helminthosporium* sp. and therefore may be difficult to obtain.

This study (Takayama et al., 2002 also provided some insight into the structure-activity relationships of mitragynine, and in two reviews (Takayama 2004; Kruegel et al., 2016) on the chemistry and pharmacology of *Mitragyna speciosa* has included more information on semi-synthetic studies of mitragynine. This structure-activity-relationship information is summarized in Figure 3 (Adkins et al., 2011).

Essentially, all the semi-synthetic derivatives had less activity than mitragynine, indicating some important structural features on the natural product. First, the 9-methoxy seems to be important for agonist activity. When the methyl ether is cleaved to the phenol, a less active agonist is produced. When the oxygen is eliminated to produce the natural product corynantheidine (3), an antagonist is produced. Thus, it is interesting that modulation of functional activity may occur at this position. It is of chemical interest that this small change to the molecule may afford templates for novel opioid antagonists, potentially with superior bioavailability to currently marketed products. Next, it also appears that disruption of the b-acrylate moiety, albeit from a very limited study, leads to less active or inactive products. Finally, loss of the basic character of the tertiary amine abolishes activity. This seems to be consistent with other opioid-based alkaloids that require a protonatable nitrogen to form a salt bridge with a conserved aspartic acid residue in transmembrane III (TM III) of the opioid receptors.

This result may indicate that mitragynine is binding in a similar mode to other known opioid ligands. However, reported comparisons of mitragynine and morphine (Takayama et al., 2002) do not indicate structural similarities, and hypothesize that mitragynine has a different binding mode than clas-

sical morphine-based ligands. It could be that some amino acid residues in the receptor are common to the affinity of both classes but some unique epitopes are involved with mitragynine recognition.

Although these studies have indicated some important structural features on mitragynine, detailed studies to elucidate the opioid pharmacophore are lacking. A few other alkaloids that have been isolated from the plant do not appear to have interesting profiles as opioid ligands, but only a few of the over 20 alkaloids have been subjected to receptor-binding studies. The C3 (quinolizidine bridge-head hydrogen) stereoisomer of mitragynine was reported to be 14-fold less active in the guinea pig ileum assay, yet no binding data was reported at opioid receptors (Takayama et al., 2002). Therefore, not all the alkaloids have been investigated nor have other non-alkaloidal ligands been characterized, and it is not entirely clear how important any of the stereochemistry is to the molecule. Moreover, no simplified analogs of mitragynine have been reported in the literature. Thus, there is a great need for a more complete understanding of the chemistry associated with *Mitragyna speciosa*.

From the reports in the literature on the naturally occurring alkaloids from *Mitragyna speciosa*, only a few of the alkaloids have been investigated for activity and some have been reported in separate studies, making them difficult to compare. More recently, the focus of literature studies has shifted to a minor component of the extract, 7-hydroxymitragynine hydroxymitragynine(2) (Ponglux et al., 1994; Matsumoto et al., 2004; Matsumoto et al., 2005). This is simply an oxidized form of mitragynine obtained from *Mitragyna speciosa*. The first report Matsumoto et al. 2004) of the *in vivo* actions of 7-hydroxymitragynine also included receptor-binding data that was obtained under the same conditions as reported for the affinities of mitragynine and 7-hydroxymitragynine. Interestingly, 7-hydroxymitragynine displayed a higher affinity to MOP than previously reported. Affinities for DOP and KOP were consistent with the previous report from the same group (Takayama et al., 2002). In this study, the tail-flick and hot-plate assays were utilized, and 7-hydroxymitragynine showed more potent effects than morphine in both tests. This is interesting since the affinity of 7-hydroxymitragynine is comparable to mitragynine and morphine. An interesting finding from this study was that 7-hydroxymitragynine was orally active and long-acting. It is now hypothesized that most of the opioid actions are a result of this compound and not mitragynine. However, the possibility of an active metabolite cannot be ruled out.

The tolerance and withdrawal symptoms of 7-hydroxymitragynine have also been studied (Matsumoto et al., 2005). In this report, the specific opioid-receptor subtypes responsible for its actions were also investigated. Tolerance developed to 7-hydroxymitragynine over time, as well as cross-tolerance to morphine. Similar to morphine, withdrawal symptoms were equally comparable upon naloxone-induced withdrawal of 7-hydroxymitragynine. It was determined that the analgesic activity of 7-hydroxymitragynine was mediated through MOP and partially through KOP. Attempts to overlay the compound with morphine were not successful, and it was concluded that 7-hydroxymitragynine may be interacting with opioid receptors in a different fashion than morphine. Overall, 7-hydroxymitragynine was shown to be a potent opioid receptor ligand that can potentially cause physical dependence.

An additional study on the effects of 7-hydroxymitragynine on gastrointestinal transit has demonstrated the involvement of MOP receptors in this action (Matsumoto et al., 2006). Interestingly, this study also investigated the receptor subtypes involved in the analgesic activity. This work came from the same group as the previous studies, and was consistent in demonstrating a MOP-selective activity. However, this time the KOP receptor was not shown to be involved. It had been previously reported by other researchers that mitragynine did not inhibit gastrointestinal transit, (Macko et al., 1972) but this study had noted limitations.

Because *Mitragyna speciosa* has been traditionally utilized to combat fatigue and promote the ability to work in harsh conditions, a study was undertaken to determine its effects on working memory (Apryani et al., 2010). This investigation involved the object-location task and the open-field test. In these paradigms, mitragynine was found to impair the cognitive function and decrease locomotion. The authors suggested this finding is similar to other mu-opioid agonists, and they further hypothesized that the memory impairment could be due to decreases in GABA neurotransmission. More studies would need to be carried out in more sophisticated paradigms to learn the effects of chronic use on working memory.

Many users of kratom, both traditional and recreational, have stated that ingestion of the plant material elevates mood and may have potential as an antidepressant. To test this idea, a study was carried out utilizing mitragynine in the mouse forced-swim test and the tail-suspension test (Idayu et al., 2011). It was determined that doses of mitragynine significantly reduced immobility in the forced-swim test and tail-suspension test, demonstrating antidepressant-like effects. Moreover,

mitragynine significantly reduced corticosterone release, which is normally elevated in stressful situations. This study showed promising potential for the use of kratom as an antidepressant, and somewhat validated the anecdotal reports of human mood elevation.

Another study was published a few years later looking into the anxiolytic-like effects of mitragynine in the open-field and elevated plus-maze paradigms (Hazim et al., 2014). These studies were done in rats, in contrast to the above study that utilized mice. This work compared the efficacy of mitragynine versus a diazepam control. The findings support the human use of kratom as an anxiolytic, where mitragynine was shown to be effective, although less than diazepam, increasing exploration in both assays. Moreover, the investigators studied three neurological systems with antagonists pre-treatment. All antagonists tested were effective in blocking mitragynine's action. This indicated that the anxiolytic-like effects are possibly due to interactions among opioidergic, GABAergic, and dopaminergic systems in the brain. It was a bit curious that serotonergic systems were not investigated in this study.

Kratom has traditionally been utilized to wean addicts off opium, and two studies have appeared in the literature investigating mitragynine and its ability to attenuate morphine withdrawal syndrome. The first study appeared utilizing zebrafish (Khor et al., 2011). Although not a commonly utilized model in opioid research, this proved to be an interesting study. Morphine was added to the water for a two-week, chronic-exposure paradigm. Mitragynine was shown to attenuate the majority of withdrawal behaviors, and real-time PCR analyses showed that it also reduced the mRNA expression of corticotropin-releasing factor receptors and prodynorphin in the zebrafish. A few years later, a study by researchers in Thailand showed an alkaloid-rich extract from *Mitragyna speciosa* was effective in attenuating naloxone-precipitated morphine withdrawal symptoms in mice (Cheaha; et al., 2017). Interestingly, their study was in direct contrast to the zebrafish study with regard to the effects of purified mitragynine. The study in mice failed to demonstrate efficacy for mitragynine alone in single-dose oral-administration studies. This is most likely due to the design of the study. To habituate the mice to morphine, doses were administered three times (50, 50, 75 mg/kg, respectively) a day for three days, and on the fourth day, mice were given a 50 mg/kg injection of morphine two hours prior to naloxone precipitation of withdrawal. In the studies that looked at ability for the alkaloid-rich extract or purified mitragynine, the mice were given only one dose of extract or mitragynine one hour after a dose of morphine and one hour prior to injection with naloxone. The only behavioral outcome that was considered for withdrawal symptoms was jumping behavior. Although various doses were examined, it is possible that a single dose of either test material would not be enough to attenuate the withdrawal effects. The authors concluded that another constituent of the alkaloid-rich extract may be responsible for the attenuation of effects seen, not mitragynine.

A group of researchers in Malaysia studied morphine-tolerance development in mice with a combination of mitragynine and morphine (Fakurazi et al., 2013). This study looked at a nine-day dosing regimen with both compounds, and evaluated the analgesic effects in the hot-plate test. Not surprisingly, the combination of morphine and mitragynine increased hot-plate latency. However, the combination of these two compounds did produce a significant reduction in morphine tolerance over morphine alone. The investigators looked at CREB-protein expression as well as liver and kidney function tests, but did not see any significant changes in the combination-treated groups. Decreasing opioid-tolerance development is hypothesized to be one way of reducing the abuse or addiction potential of these agents, and certainly a way to avoid some of the side effects that can arise from increasing dosing regimens.

Another way to examine the abuse or addictive potential of opioids is through the conditioned place preference (CPP) paradigm. Two reports have appeared in the literature that are in overall agreement demonstrating that mitragynine causes a place preference, and that indicate a drug-seeking behavior is associated with mitragynine. It is interesting that the first study failed to demonstrate a statistically significant place preference for an extract of *Mitragyna speciosa*, but did show each dose of purified mitragynine to cause a place preference (Sufka et al., 2014). It is important to note that the error bars were quite large in these studies. The second study looked at CPP in a bit more detail, and looked directly at opioid-receptor involvement by the use of antagonists (Yusoff et al., 2017). This study was aimed at investigating whether the reinforcing effects of mitragynine were mediated by opioid receptors. This study demonstrated that naloxone was effective at blocking mitragyine CPP overall. Interestingly, the investigators also showed that the acquisition, but not the expression, of mitragynine place preference is mediated through opioid receptors. These findings add more support to the mu-opioid agonist activity of mitragynine.

Finally, an investigation was carried out to determine the discriminative stimulus properties of mitragynine in rats (Harun et al., 2015). Drug discrimination is a valid paradigm to compare sub-

stances, especially psychoactive ones. This work utilized rats that were trained to discriminate morphine from vehicle. Interestingly, the investigators also wanted to examine the stimulant properties (thought to be a result of mitragynine) by using a group trained with cocaine. Rats were able to discriminate between mitragynine and saline, similar to another group that was able to discriminate between morphine and saline. The dose required for mitragynine was 3-fold higher than that of morphine. Both mitragynine and 7-hydroxymitragynine individually substituted completely to morphine discriminative stimulus. This suggests that there is pharmacological similarity between the two compounds. Interestingly, mitragynine also partially generalized to cocaine discriminative stimulus. Thus, both the opioid- and stimulant-like effects of *Mitragyna speciosa* can potentially be due to the major alkaloid, mitragynine.

All of the studies that have been conducted have shown that mitragynine and 7-hydroxymitragynine are opioid-receptor agonists. However, there are inconsistencies (as pointed out above) in these studies, making the interpretation of the entire body of pharmacological literature difficult to fully understand the pharmacology and chemistry associated with these compounds. Mitragynine and 7-hydroxymitragynine have been shown to work through opioid mechanisms *in vivo* and *in vitro* (Babu et al., 2008; Akins et al., 2011). They have also been shown to have activity in the serotonin and adrenergic systems (Matsumoto et al., 1996). This is not surprising upon review of the structure, which contains the tryptamine nucleus. One study that is completely lacking in the literature is the ability of mitragynine or 7-hydroxymitragynine, or extracts of *Mitragyna speciosa*, to be self-administered. Such a study would provide the most solid evidence for the abuse/addiction liability associated with the individual alkaloids or extracts. The fact that mitragynine alone interacts with multiple targets in the CNS, not to mention the full extracts' multiple activities, certainly helps to explain why the pharmacology is complex. However, it may be that this complex pharmacology has provided a natural antidote to opioid addiction. This remains to be seen, at least from a scientifically sound study and carefully controlled human clinical trial. There remains a great deal of scientific work that needs to be carried out on this plant species and its constituents to determine the chemical and pharmacological reasons that it is used traditionally and recreationally, to anticipate potential toxicities and potential therapeutic components. There seems to be great therapeutic promise to kratom.

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