CROSS-REINSTATEMENT MODELS OF MITRAGYNINE-MORPHINE DRUG-SEEKING ADDICTIVE BEHAVIOUR AND DOPAMINERGIC INVOLVEMENT IN SPRAGUE-DAWLEY RATS

RIMA ATRIA JAPARIN

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by

RIMA ATRIA JAPARIN

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LIST OF SYMBOLS

°C	degree celcius
%	percent
-	minus
±	plus minus
x	multiply
=	equals to
/	per (for each)
μ	micro
<	less than
>	more than
Δ	delta

LIST OF ABBREVIATIONS

AADK	Agensi Anti Dadah Kebangsaan
ANOVA	Analysis of variance
CNS	central nervous system
CPP	conditioned place preference
CRF	corticotropin-releasing factor
DEA	Drug Enforcement Administration
FDA	Food and Drug Administration
i.p.	intraperitoneal
p.o.	By oral
i.e.	That is
e.g.	For example
et al.	and others
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
FR	Fixed ratio
CB-1	Cannabinoid-receptor-1
CPu	Caudate-putamen
DA	Dopamine
DAT	Dopamine transporter
DOPAC	3,4-dihydroxyphenylacetic acid
DORs	delta-opioid receptors
DRRF	dopamine receptor-regulating factor
GABA	gamma-aminobutyric acid
HVA	homovanillic acid
IVSA	intravenous self-administration
ICSS	intracranial self-stimulation
KORs	kappa-opioid receptors
LC	Locus coeruleus

mg/kg	milligram per kilogram
MORs	mu-opioid receptors
MGM-9	ethylene glycol-bridged and C10-fluorinated derivative of
MMT	Mitragynine
	Methadone maintenance treatment
mRNA	Messenger ribonucleic acid
NA	noradrenaline
NAc	nucleus accumbens
OUD	opioid use disorder
PFC	prefrontal cortex
RM	Ringgit Malaysia
SDN BHD	Sendirian berhad
SEM	standard error of means
THC	Δ-9-tetrahydrocannabinol
UNODC	United Nations Office on Drugs and Crime
USA	United States of America
VP	Ventral pallidum
VTA	Ventral tegmental area

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MODEL PENGEMBALIAN SILANG BAGI TINGKAH LAKU KETAGIHAN PENCARIAN DADAH MITRAGININA-MORFINA DAN PENGLIBATAN DOPAMINERGIK DALAM TIKUS SPRAGUE-DAWLEY

ABSTRAK

Kratom (*Mitragyna speciosa* Korth.) merupakan sejenis tumbuhan herba ubatan yang semakin popular sebagai pengganti opioid. Walau bagaimanapun, tidak banyak yang diketahui mengenai potensi penyalahgunaan alkaloid utamanya, mitraginina, terutamanya dalam ketagihan semula drug (relaps). Oleh itu, model-model kepupusan pengembalian semula seperti paradigma-paradigma kecenderungan tempat berkondisi (CPP) dan pemberian sendiri drug intravena (IVSA) dijalankan untuk mengimitasi mekanisme ketagihan semula dalam tikus Sprague-Dawley. Dalam bahagian pertama kajian, mitraginina diberikan secara bukan kontinjen kepada tikus ketagihan morfina selepas latihan kepupusan. Berikutan fasa pemerolehan CPP teraruh mitraginina (30 mg/kg, i.p.) atau morfina (10 mg/kg, i.p.), tikus menjalani latihan kepupusan. CPP teraruh mitraginina kembali semula berikutan suntikan morfina (1, 3 dan 10 mg/kg, i.p.). Pada masa yang sama, CPP teraruh morfina kembali semula berikutan suntikan mitraginina (3, 10 and 30 mg/kg, i.p.). Dalam kajian menggunakan tatacara pengambilan sendiri drug intravena, tikus-tikus dilatih untuk mengambil sendiri morfina (0.5 mg/kg/infusi) secara intravena di bawah nisbah tetap (FR-3) dalam jadual pengukuhan. Penyingkiran kedua-dua infusi morfina dan isyarat berkaitan drug menyebabkan kepupusan tingkah laku mencari drug. Ujian-ujian pengembalian semula dilakukan selepas suntikan mitraginina (3, 10 dan 30 mg/kg), morfina (5 mg/kg) dan pembawa yang diberi secara rawak. Suntikan mitraginina pada dos 10 mg/kg menyebabkan gerak balas tuil tekan pengambilan sendiri morfina untuk kembali semula tetapi dos tertinggi mitraginina (30 mg/kg)

mengurangkan gerak balas tuil tekan daripada pemberian sendiri morfina. Untuk memahami mekanisme yang terlibat dalam sifat-sifat ganjaran mitraginina dalam konteks ketagihan semula, penglibatan sistem dopamina (DA) dalam fasa pemerolehan, pengekspresan dan pengembalian semula turut dikaji. Maka, bahagian kedua kajian ini dijalankan menggunakan antagonis selektif reseptor DA D1, SCH-23390. Dalam fasa pemerolehan CPP, tikus-tikus diberi pra-rawatan dengan SCH-23390 (0, 0.1 and 0.3 mg/kg, i.p.) sebelum dikondisikan dengan mitraginina (10 mg/kg). Seterusnya, kesan-kesan antagonis reseptor DA D1 terhadap fasa pengekspresan CPP teraruh mitraginina diuji. Kemudian, kesan-kesan suntikan mitraginina (5 mg/kg) terhadap pengembalian semula CPP teraruh berikutan kepupusan dijalankan. Keputusan menunjukkan bahawa SCH-23390 menghalang pemerolehan CPP teraruh mitraginina tetapi tidak menghalang pengekspresan CPP teraruh mitraginina. Tambahan pula, reseptor DA D1 yang dihalang ketika fasa kondisi tidak menyekat kesan-kesan suntikan mitraginina terhadap ujian pengembalian semula dalam CPP dan ini mencadangkan bahawa reseptor DA D1 tidak memainkan sebarang peranan dalam sensitiviti pengembalian semula. Kesimpulannya, keputusan-keputusan kajian ini menunjukkan bahawa pendedahan kepada mitraginina menggalakkan peningkatan kadar ketagihan semula dan mencadangkan bahawa potensi mitraginina sebagai ubat pengawalan opioid memerlukan kajian saintifik yang lebih lanjut dari segi ketagihan semula ke penggunaan opioid.

CROSS-REINSTATEMENT MODELS OF MITRAGYNINE-MORPHINE DRUG-SEEKING ADDICTIVE BEHAVIOUR AND DOPAMINERGIC INVOLVEMENT IN SPRAGUE-DAWLEY RATS

ABSTRACT

Kratom (*Mitragyna speciosa* Korth.) is a medicinal herb which gained fame for its potential as an opioid substitute. Nevertheless, little is known about the abuse potential of its major alkaloid, mitragynine, especially in relapse to drug abuse. Therefore, the extinction-reinstatement models including the conditioned place preference (CPP) and intravenous self-administration (IVSA) paradigms, were employed to model the relapse mechanism in Sprague-Dawley rats. In the first part of the study, mitragynine administered non-contingently in morphine-addicted rats following extinction was investigated. Following CPP acquisition induced by either mitragynine (30 mg/kg, i.p.) or morphine (10 mg/kg, i.p.), rats were subjected to repeated CPP extinction sessions. A priming injection of morphine (1, 3 and 10 mg/kg, i.p.) dose-dependently reinstated mitragynine-induced CPP. Similarly, a priming injection of mitragynine (3, 10 and 30 mg/kg, i.p.) dose-dependently reinstated morphine-induced CPP. In the IVSA study, rats were initially trained to intravenously self-administer morphine (0.5 mg/kg/infusion) under a fixed ratio-3 schedule of reinforcement. Removal of both morphine infusions and drug-associated cues led to the extinction of drug-seeking behaviour. Reinstatement tests were made following a randomised order of mitragynine (3, 10 and 30 mg/kg), morphine (5 mg/kg) and vehicle injections. Mitragynine priming at 10 mg/kg resulted in the reinstatement of morphine-seeking behaviour but higher mitragynine dose (30 mg/kg) suppressed the seeking response. In order to understand the mechanism underlying the rewarding properties of mitragynine in relapse, the involvement of dopaminergic

system in the acquisition, expression and reinstatement phase was studied. Therefore, the second part of the study was conducted using a selective dopamine (DA) D1 receptor antagonist, SCH-23390. For acquisition, rats were pre-treated with SCH-23390 (0, 0.1 and 0.3 mg/kg, i.p.) prior to mitragynine (10 mg/kg) conditioning sessions. Next, the effects of DA D1 receptor antagonist were tested on the expression of mitragynine-induced CPP. Subsequently, the effects of a mitragynine-priming dose (5 mg/kg) on the reinstatement of extinguished CPP were tested. The results showed that SCH-23390 dose-dependently suppressed the acquisition of mitragynine-induced CPP but no effect on the expression of mitragynine-induced CPP. Additionally, blockade of the D1-like receptor during conditioning did not prevent mitragynine priming effects in CPP reinstatement test, implying no role of the DA D1 receptor in reinstatement sensitivity. Altogether, these findings suggest that exposure to mitragynine may increase the likelihood of relapsing to opioids, suggesting that mitragynine's potential as an opioid management treatment merits further scientific assessment of its ability to trigger relapse to opioid abuse.

CHAPTER 1
GENERAL INTRODUCTION

1.1 An overview of the study

Opioid use disorder (OUD) is associated with misuse of prescription opioid medications or use of illicitly obtained opioids (i.e. morphine, heroin and fentanyl) (Volkow et al., 2019; Strang et al., 2020) which is a chronic, relapsing condition. Opioid misuse is a major health problem with a significant impact on society. As per the United Nations Office on Drugs and Crime (UNODC), roughly 296 million individuals, or 5.8% of the global population between the ages of 15 to 64 years, take drugs at least once in 2021. Among them, an estimated 60 million use opioids (United Nation Office on Drugs and Crime, 2023). There is apprehension regarding the worsening opioid epidemic with the increase in opioid-related mortality cases (Ciccarone, 2021). Provided that OUD treatments such as methadone and buprenorphine are typically associated with compliance issues, abuse potential, negative side effects and a high risk of relapse (Gonzalez et al., 2004; Volkow et al., 2016), it is necessary to search for medication that is safer and more effective. Thus, the circumstance calls for an urgent need for safer alternative OUD medications.

Mitragyna Speciosa Korth. (of the Rubiaceae family) or kratom is a medicinal tree native to Southeast Asia especially in Malaysia and Thailand, with vast medicinal properties (Adkins et al., 2011; Hassan et al., 2013). At present, kratom is gaining worldwide popularity as an opioid-like herbal medicine for its pain-relieving effects and treatment of psychiatric problems in addition to opioid dependence and withdrawal (Singh et al., 2017; Anand & Hosenagar, 2022). Kratom is traditionally consumed either by chewing the leaves fresh or preparing tea from

leaves (Hassan et al., 2013). On the other hand, in Western countries, kratom is accessible as powder, pills, capsules or concentrated extracts which can be acquired both online and in street shops (Boyer et al., 2008; Prozialeck et al., 2012; Scott et al., 2014; Lydecker et al., 2016; Guddat et al., 2016; Sharma et al., 2019). Apart from the benefits of kratom, its usage has been linked to the development of dependence, tolerance and withdrawal (Vicknasingam et al., 2010; Singh et al., 2014; Saingam et al., 2014; 2016). According to Singh et al. (2014), there was a moderate level of craving for kratom indicated by users, and after three months of abstinence, a significant proportion of kratom users (78 - 89%) experienced a relapse (Singh et al., 2014; 2015; Vicknasingam et al., 2010). Furthermore, it is noteworthy that kratom appears to be used by opioid poly-drug users as a preventative measure against relapsing into opioid use, which has led to the rise in popularity of kratom as an alternative among users who are dependent on the use of both illegal and prescription opioid (Singh et al., 2014; Grundmann, 2017; Swogger & Walsh, 2018). Therefore, the disparity in the aforementioned findings stipulates further research in the assessment of kratom relapse and its relapse to opioid, particularly the plant extracts containing active compounds.

Furthermore, pre-clinical findings also discovered that the most probable mechanism underpinning the neuropharmacological basis of kratom use by opioid users is its interaction with the opioid system (Harun et al., 2015, 2022). Alkaloids, particularly mitragynine, are thought to be responsible for the pharmacological effects of kratom. Mitragynine is the main active constituent of kratom, which varies between 12% to 66% of the total amount of alkaloids present in the kratom plant extract, in accordance with its geographical origin (Suhaimi et al., 2016; Ponglux et al., 1994; Hassan et al., 2013). Other factors affecting mitragynine content include

seasonal changes and age of plants (Leon et al., 2009; Pearson et al., 2018; Karunakaran et al., 2022). Several pharmacological effects of mitragynine were shown to resemble those of opioids, especially morphine (Harun et al., 2015, 2020; Yusoff et al., 2016). The findings which show that mitragynine has low abuse potential (Henningfield et al., 2018), could reduce morphine self-administration (Hemby et al., 2019) and is not associated with respiratory depression compared with opioids (Chinnappan et al., 2023) point to the possibility that mitragynine may be used as a less harmful opioid substitute for managing OUD. However, the possibility remains that mitragynine may exert effects which could trigger relapse to opioid use based on pre-clinical (Harun et al., 2015; Yusoff, 2018b) and clinical studies (Boyer et al., 2008; White, 2018; Müller et al., 2020; Singh et al., 2022), a concern that has not been addressed.

The extinction-reinstatement model is regarded as a measure of relapse in laboratory animals where it involves the establishment and consequent extinction of a learned behavioural response (Aguilar et al., 2009). The extinction-reinstatement models that are frequently utilised include the conditioned place-preference (CPP) and intravenous self-administration (IVSA) (Ahsan et al., 2014). The CPP model has been employed to evaluate addiction-related behaviours and for the quantification of rewarding effects or hedonic value of the drug of interest (Carboni et al., 1989; Ahsan et al., 2014). The IVSA paradigm, on the other hand, is regarded as the ‘gold standard’ animal model of addiction which measures drug-seeking and drug-taking behaviour (reinforcing effects) (Vastola et al., 2002; Ahsan et al., 2014).

Furthermore, the dopamine (DA) release and the activation of DA receptors in the nucleus accumbens (NAc) are well-known mechanisms by which drugs of abuse produce their rewarding effects (Di Chiara & Imperato, 1988). DA acts on DA

D1- and D2 receptors. While D1-like receptors are involved in reward-related learning during the formation of stimulus–response associations, D2-like receptors play an important role in magnifiying the motivational salience of conditioned rewards after the occurrence of learning (Beninger & Miller, 1998). Based on a recent study, mitragynine markedly increased DA release and dopamine transporter (DAT) mRNA expression following its repeated administration in anesthetized rats (Effendy et al., 2021). In contrast, a microdialysis study showed that acute mitragynine exposure did not result in a significant increment in extracellular DA activity. Instead, there was a significant increment in DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) in the caudate-putamen (CPu) and homovanillic acid (HVA) in the NAc, CPu and prefrontal cortex (PFC) in rats, indicating a strong and prolonged effect of mitragynine on DA synthesis and/or metabolism, but not on extracellular DA activity (Yusoff et al., 2022). The disparity in findings may be due to the effects of dopaminergic transmission, which may become apparent after repeated mitragynine treatment when an earlier report has implicated that subchronic mitragynine administration could alter DA receptor and DAT expression in the mesolimbic system of mice (Effendy et al., 2021). Although these findings suggest a DA receptor-mediated mechanism of mitragynine reinforcement, the contribution of specific DA receptor subtypes that may be involved in relapse remains vague.

Hence, the first part of the current study aims to evaluate the reinstatement effects of mitragynine on opioid-seeking behaviour using both the extinction-reinstatement models which include the CPP and IVSA paradigms. Meanwhile, the second part of the present study is aimed to characterize the role of the DA subtype, particularly the DA D1 receptor, in the rewarding effects of mitragynine. The

employment of both extinction-reinstatement models (i.e. CPP and IVSA paradigms) in this study is pertinent to comprehend the addictive properties of mitragynine related with drug relapse. Therefore, the results obtained from the aforementioned behavioural models could offer a thorough comprehension of how mitragynine could be developed as a potential pharmacotherapeutic intervention in treating OUD. Additionally, a better understanding of the underlying mechanism (i.e. DA system) of the rewarding properties of mitragynine could contribute to its use as an OUD treatment.

1.2 Problem statements

Multiple findings are highlighting the use of kratom in the management of OUD (Boyer et al., 2007; Vicknasingam et al., 2010; Prozialeck et al., 2012; Singh et al., 2016). Research has indicated that individuals in Southeast Asia (Vicknasingam et al., 2010) and the United States (US) (Boyer et al., 2008; Coe et al., 2019; Grundmann, 2017) have used kratom as a means to curb opioid withdrawal symptoms and as an opioid substitute (Garcia-Romeu et al., 2020). Of interest is the finding that current opioid poly-drug users are in need of continued kratom use to prevent their return to opioid use (Singh et al., 2020). However, it is still unknown in terms of the neurobiological mechanisms involved in kratom use as a substitution treatment amongst opioid addicts. This warrants further investigation into the role of kratom in opioid relapse.

Additionally, despite numerous findings suggesting the potential of kratom's active ingredient, mitragynine as an opioid alternative (Khor et al., 2011; Harun et al, 2015; Hassan et al., 2020; Wilson et al., 2021; Harun et al., 2022), studies on the

reinstatement effects of mitragynine in animal models have not yet been conducted. Therefore, it is of interest to know whether mitragynine could reinstate opioid-seeking behaviour following extinction and vice-versa, using animal models of relapse. It is hypothesised that mitragynine may induce opioid craving and precipitate relapse to opioid-taking behaviour.

Furthermore, the dopaminergic pathway is believed to be involved in mediating the rewarding properties of mitragynine. It is a well-established fact that drugs of abuse including opioids significantly increases the mesolimbic dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) which results in the reward-related effects (Spanagel &Weiss, 1999). Two receptor classes are involved in the dopaminergic pathway which includes D1- and D2-like receptors (Missale et al., 1998; Zarrindast et al., 2003). The findings of kratom binding affinity to DA receptors research are conflicting. Although its ability to bind to DA D2 receptors has been reported (Boyer et al., 2008), another study discovered that kratom only binds to DA D1 receptors with low affinity (Stolt et al., 2014). Therefore, the mechanisms underlying DA receptor-mediated rewarding effects of mitragynine that could be-mediated by a particular DA receptor subtype remains inadequate. Hence, investigation on the role of the DA D1 receptors in mediating the acquisition, expression and reinstatement of mitragynine-induced CPP was tested in the present study. It is hypothesised that the dopaminergic receptor system is involved in the pharmacological mechanisms in mitragynine-induced CPP.

1.3 Significance of the study

This study is essential to gain more insight into the addictive behaviour of mitragynine, particularly in the drug relapse phenomenon, through the aid of animal models. In addition, this research would be beneficial in addressing the disputes surrounding the legal status of kratom both domestically and internationally. The present study would also facilitate the understanding of neurobiological mechanisms which underlie kratom's use as a substitution treatment for opioid addicts, paramount in the establishment of mitragynine or kratom as a cost-effective opioid substitution therapy. Consequently, substantial economic gains could be obtained with decreased costs for national health care if OUD could be effectively managed.

1.4 Objectives of the study

The general objective is to determine relapse in mitragynine, in rats. The specific objectives of the current study are listed below:

i) To assess the effect of mitragynine on reinstatement of morphine-induced CPP and the effect of morphine on reinstatement of mitragynine-induced CPP.

ii) To examine the ability of mitragynine doses to reinstate morphine-seeking behaviour in rats through the intravenous self-administration (IVSA) paradigm.

iii) To determine the effects of DA D1 antagonist SCH-23390 on the acquisition and expression of mitragynine-induced CPP.

iv) To evaluate the effects of DA D1 antagonist SCH-23390 administered during the acquisition phase on the reinstatement of an extinguished mitragynine-induced CPP.

1.5 Summary of the research design

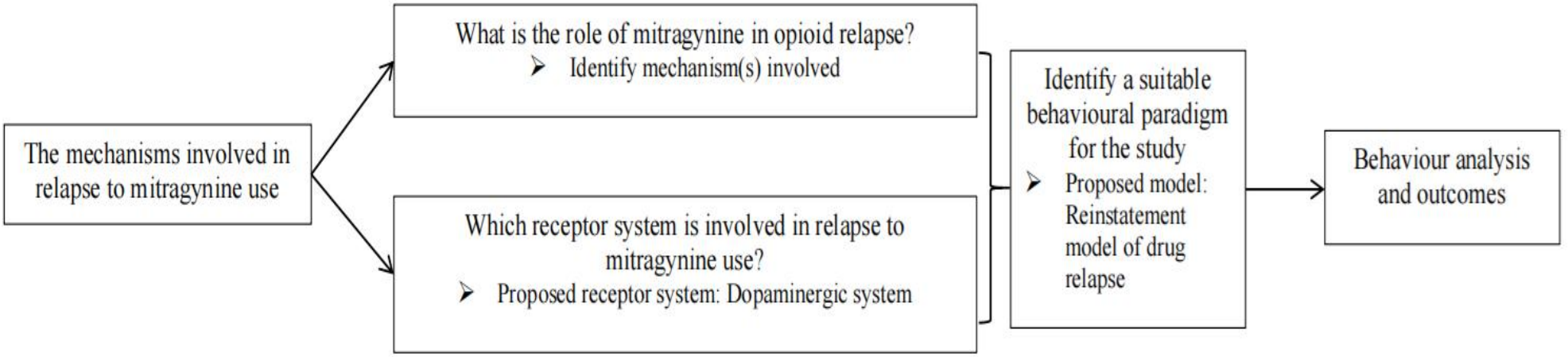

Figure 1.1 A conceptual framework of the study

CHAPTER 2
LITERATURE REVIEW

2.1 *Mitragyna speciosa* Korth.

2.1.1 Botanical origin

Mitragyna speciosa Korth. is a tree-like plant endemic to Southeast Asia, specifically Malaysia and Thailand, where it is known as 'ketum' or 'biak-biak' and 'kratom', 'kakuam', 'kraton', 'ithang' or 'thom' respectively (Jansen & Prast, 1988; Adkins et al., 2011; Ramanathan & McCurdy, 2020). Often referred to as "kratom" (a colloquial term used to describe both the plant and the botanical products derived from its leaves), *M. speciosa* belongs to the *Rubiaceae* family ('coffee' family) (Davis, 2006; Suhaimi et al. 2016; Eastlack et al., 2020). The *M. speciosa* tree usually grows to a normal height of 4-9 meters (13-30 feet) and 5 meters (16 feet) wide although some species could reach a height of up to 15-30 meters (50-98 feet) (Shellard & Lees, 1965; Shellard, 1974; Hassan et al., 2013). The plant thrives in fertile and humus-rich soil with sufficient sunlight in locations shielded from strong winds (Macko et al., 1972; Hassan et al., 2013). The leaves are dark green and glossy which are ovate-acuminate in shape with tapered ends and may reach 14-20 cm (5.5-7.9 inches) in height and 7-12 cm (2.8-4.7 inches) wide (Eisenman, 2014). *M. speciosa* leaves grow with opposite arrangement with 12 to 17 pairs of veins (Shellard & Lees, 1965; Hassan et al., 2013; Eisenman, 2014).

The leaves have either green or red where the latter is preferred due to its potency (Suwanlert 1975; Saingam et al., 2013; Singh et al., 2017; Meireles et al., 2019). Meanwhile, the local people in southern Thailand presume that red-veined leaves possess medicinal benefits while the green-veined leaves are useful to increase

energy levels (Nakaphan et al., 2016; Sengnon et al., 2023). The estimated weight range of a fresh leaf is from 1 to 4 g, while a dry leaf is about 0.43 g (Suwanlert 1975; Sengnon et al., 2023). In the dry season, leaves fall profusely but new growth is expected during the rainy season (Macko et al., 1972). The stem is upright and branched, bearing globular-shaped dark yellow flowers (could bear up to 120 florets each) which occur in clusters of three at the ends of the long stalks (Jansen & Prast, 1988; Hassan et al., 2013; Singh et al., 2016). The leaves and smaller stems of the trees are typically used for consumption. Images of the *M. speciosa* plant are as illustrated in Figure 2.1.

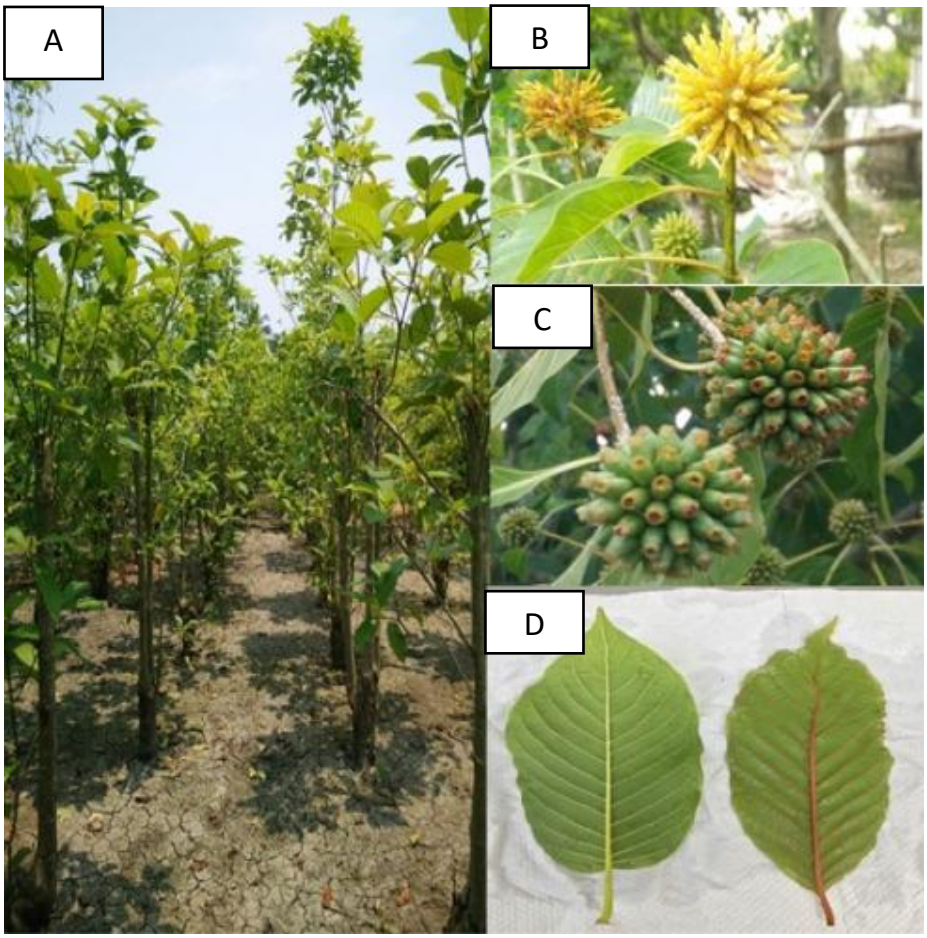

Figure 2.1 The morphology of the *M. speciosa* plant (A) represents the tree, (B) and (C) represent the inflorescence and (D) represents the leaves. Pictures are adopted from Ramanathan et al. (2021).

2.1.2 Plant preparation and consumption

Kratom can be consumed through various modes where the leaves could be smoked, chewed, made into herbal tea or combined with beverages such as coffee (Hassan et al., 2013; Singh et al., 2017). In Malaysia, kratom is often ingested as a solution (also called tea or juice) derived from boiled *M. speciosa* leaves (Singh et al., 2014). Kratom vendors would often favour purchasing older leaves from farmers to prepare kratom juice. Fresh kratom leaves would be rinsed with water to get rid of any remaining dirt prior to boiling in a pot of water. Soon afterward, the leaves

would be brewed at a lower temperature for up to 4 hours and stirred on a half-hourly basis to make certain the leaves do not get attached to the pot's surface. The juice production is complete when it releases a strong odour. The kratom juice would be allowed to cool before being sold in small, clear plastic bags. Storing the packets in ice would enable them to last up to 3 days (Singh et al., 2017). In Malaysia, the typical price for each kratom juice pack (between 250 and 300 ml of fresh kratom juice) is RM5 (USD = 1.20; Singh et al., 2014). It is acquired easily from local illegal distributors of kratom. Sweeteners and flavour enhancers such as sugar and honey are sometimes added to kratom tea to mask its unpleasant and bitter taste (Hassan et al., 2013; Singh et al., 2017; Henningfield et al., 2018).

Meanwhile, in Thailand, it is common for kratom users to chew on fresh kratom leaves (Suwanlert, 1975; Singh et al., 2017) multiple times each day, as needed (Suwanlert, 1975). In the last few years, homemade kratom cocktail known as '4x100' (i.e. sii khoon roi), has been gaining popularity among the youth in Thailand. The cocktail is a combination of boiled *M. speciosa* leaves, caffeinated soft drinks including Coca-Cola and cough syrup which contains either codeine or diphenhydramine (Tungtananuwat & Lawanprasert, 2010; Tanguay, 2011; Hassan et al., 2013; Singh et al., 2017). Apart from the aforementioned ingredients, users may add an array of substances such as anxiolytics, analgesics and antidepressants, sleeping pills, powdered mosquito coils, pesticides and rat poison based on their preferences (Tungtananuwat & Lawanprasert, 2010; Saingam et al., 2013; Ramanathan & Mansor, 2014; Singh et al., 2017). It is believed that this consumption method would maximise both the absorption of the alkaloids in their unionized state and the effects of kratom (i.e. sedative and euphoric effects) (Tanguay et al., 2011). The use of 4 × 100 with poly-drugs (anxiolytic or antidepressant) can cause fatality

because of its multidrug toxicity (Tungtananuwat & Lawanprasert, 2010; Singh et al., 2017). In fact, a similar trend referred to as koroi (i.e. kratom juice, cough syrup and Coca-cola), has attained notoriety among Malaysian users, both young and old.

Over the past decades, kratom use became widespread and has increased substantially in the West. According to anecdotal reports, Southeast Asian immigrants were believed to be responsible for the importation of kratom into the United States during 1980s and 1990s (Kruegel, & Grundmann, 2018; Henningfield et al., 2018; Veltri & Grundmann, 2019). Unlike kratom use in Southeast Asia, kratom tree and its fresh leaves are not readily available for consumption in the West (Henningfield et al., 2018). Hence, kratom could be found in the form of pills (i.e. capsules, tablets), extracts, gums and leaves for brewing or chewing (Boyer et al., 2007; Warner et al., 2016) or blended products (i.e. with hemp or kava) (Brown et al., 2017; Ramanathan & McCurdy, 2020) on web-based pharmacies (i.e. internet), head shops or herbal stores. Kratom products could also be seen marketed as an herbal medicine or supplement and given the nickname “herbal speedball” (Prozialeck et al., 2012; Cinosi et al., 2015; Singh et al., 2016; Grundmann, 2017; Drug Enforcement Administration, 2017; Veltri & Grundmann, 2019). In the United States, users would usually opt to consume kratom in the form of tea using kratom powder. Acids have been added to enhance the extraction while the bitter taste of tea is reduced with sweeteners, sugar or honey (Henningfield et al., 2018; Veltri & Grundmann, 2019). To produce a stronger euphoria, kratom is reportedly combined with other substances such as opioids, ethanol, stimulant, benzodiazepines, cough syrup containing codeine or diphenhydramine and quetiapine (Kronstrand et al., 2011; Post et al., 2019; Gershman et al., 2019; Hughes, 2019; Davidson et al., 2021).

2.1.3 Uses of kratom

Historically, kratom use has become a long-standing commonplace particularly among the Southeast Asian rural populations for medicinal purposes. It has been used to alleviate minor illnesses such as fever, diarrhoea, diabetes, diarrhoea, muscle aches and coughing. Additionally, it promotes wound healing (Hassan et al. 2013; Singh et al., 2016). Aside from its therapeutic values, male manual labourers (i.e. fisherman, farmers, rubber-tappers) would consume kratom leaves for both their stimulant and opium-like effects to improve physical stamina and strength as well as a way for stress relief (Suwanlert, 1975, Assanangkornchai et al., 2007; Vicknasingam et al., 2010; Ahmad & Aziz, 2012; Singh et al., 2016). Interestingly, kratom is also served as a recreational drink for social gatherings (Ramanathan & McCurdy, 2020). Muslims in Malaysia and South Thailand use kratom in place of alcohol as alcohol consumption is forbidden in Islamic teachings (Tungtananuwat & Lawanprasert, 2010; Tanguay, 2011; Singh et al., 2017). Moreover, kratom users are not subjected to the social stigma associated with alcohol consumption because there are not viewed as alcohol users (Saingam et al., 2013; Singh et al., 2017). In addition, some have claimed that *M. speciosa* consumption could boost libido (Vicknasingam et al., 2010; Singh et al., 2016).

According to Burkill (1935), reports indicate that the use of kratom as an inexpensive opium alternative in Malaya has been noted as early as 1836 during opium shortages (Hassan et al., 2013; Cinosi et al., 2015). According to a survey, most of the kratom users in the northern regions of Peninsular Malaysia (90%) depended on the plant as an affordable substitute to reduce the use of opiates and other drugs such as cannabis and amphetamine-type stimulants while 84% of the users noticed an added benefit in ameliorating opioid withdrawal (Vicknasingam et

al., 2010). Furthermore, most of the Malaysian kratom users had previously used drugs (Vicknasingam et al., 2010; Ahmad & Aziz, 2012; Saingam et al., 2013). It is worth noting that current opioid poly-drug users in Malaysia consume kratom to alleviate the symptoms of opioid withdrawal while kratom is used by former opioid poly-drug users for its mood-enhancing properties to abstain from opioid use (Singh et al., 2020). Moreover, a recent study has reported that kratom is consumed by users who co-administer heroin and methamphetamine by reducing the intake of these substances or by substituting them (Singh et al., 2022), which indicates that kratom is also used to reduce methamphetamine intake.

Due to the commercial formulations of kratom in recent years, it has become easily accessible to users worldwide (Eastlack et al., 2020) and its prevalence has been rising rapidly especially in Europe and the United States (Forrester, 2013; Nizar et al., 2015; Eastlack et al., 2020). Kratom was initially used as an herbal treatment for chronic pain in the West. Thereafter, it was viewed as a frugal substitute for self-management of pain during opioid withdrawal (Boyer et al., 2007, 2008; McWhirther & Morris, 2010; Nelsen et al., 2010; Neerman et al., 2013; McIntyre et al., 2015; Singh et al., 2016) or even heroin, suboxone (Cinosi et al., 2015) and alcohol (Singh et al., 2016.) withdrawal symptoms. According to online surveys conducted in the United States, kratom users reported an increased level of energy, reduced pain, improved concentration, decreased anxiety levels, less depressed mood, reduced or discontinued use of opioid painkillers, reduction of post-traumatic stress disorder (PTSD) symptoms and elevated mood (Grundmann, 2017; Veltri & Grundmann, 2019; Schimmel et al., 2021). Kratom is involved in poly-drug use or as a substitute for prescription opioids or heroin in the United States (Smith et al., 2023).

2.1.4 Legality and regulation of kratom

M. speciosa use has been banned in Malaysia since the government gazetted kratom into the First and Third Schedules of the Poisons Act of 1952 in 2003 (Hassan et al., 2013; Khalil et al., 2020). Although cultivation of kratom trees is not deemed illegal, anyone found guilty of possessing processed kratom leaves or distributing kratom would be punished by imprisonment which does not exceed 5 years or fines not exceeding RM100,000, or both (Pharmaceutical Services Programme, 2024). Despite legal attempts to have kratom classified as poisonous through the enforcement of the Poisons Act of 1952 (Khalil et al., 2020), the level of kratom misuse has not abated. Therefore, several organizations such as the Malaysian Drug Prevention Association (Pemadam) (Borneo Post, 2015) and the Malaysian Substance Abuse Council (MASAC) (New Straits Times, 2023) have prompted the government to reschedule kratom use under the Dangerous Drugs Acts, 1952 which will carry heavier penalties.

Meanwhile, Thailand was the first to regulate the use of kratom through the Kratom Act since 1943 which made it unlawful to plant, possess and import or export kratom leaves (Drug Enforcement Administration, 2013; Narsa, 2022). Subsequently, the Thai government reclassified kratom under the less restrictive Thai Narcotics Act in 1979, which replaced the former Kratom Act of 1943. However, Thailand updated its Narcotics Act in August 2021 which removed kratom from the list of level 5 narcotic substances (Sengnon et al., 2023). As a result, kratom-related offences such as the possession and consumption of kratom were decriminalised. On 26th August 2022, the Thai government gazetted a new law, the Kratom Plant Act, where it is designed to improve economic activity related to kratom, by allowing the online trade of kratom-based products such as foods and drinks. However,

regulations and restrictions on the trade are still imposed by the government (Bangkok Post, 2022).

Western countries have differing legal standing on kratom. To date, kratom is not placed under the US Controlled Substances Act (CSA) under scheduling although the US Drug Enforcement Administration (DEA) does not acknowledge any legitimate therapeutic use of kratom (Drug Enforcement Administration, 2013; Veltri & Grundmann, 2019). In a letter to the Drug Enforcement Agency (DEA) in 2017, the Department of Health and Human Services (HHS) proposed that kratom should be classified as a Schedule I controlled substance under the CSA (Ramirez et al., 2021). The letter included admonitions from both the United States Food and Drug Administration (FDA) and the National Institute on Drug Abuse (NIDA) that kratom should be included as a Schedule I drug under CSA (Ramirez et al., 2021). However, kratom remains listed under the 'Drugs and Chemicals of Concern' under the DEA which only describes kratom as a substance of potential abuse and danger (Drugs of Concern List, 2020; Ramirez et al., 2021). Regardless of the federal government's stance on kratom, there is disparity in kratom legislation from state-to-state (Ramirez et al., 2021). Kratom is currently legal in 44 states and is banned in the following six states: Rhode Island, Vermont, Alabama, Arkansas, Wisconsin and Indiana (Ramirez et al., 2021).

Meanwhile, kratom is classified as a "psychoactive substance" by the Psychoactive Substances Act 2016 in the United Kingdom. As a result, the act makes it an offense to produce, supply and trade kratom in the country (Psychoactive Substances Act, 2016). On the other hand, kratom is illegal in different parts of Europe which include Finland, Iceland, Latvia, Lithuania, Poland, Denmark, Sweden, Romania and France, among others (Veltri & Grundmann, 2019).

2.1.5 Phytochemistry

Extensive phytochemical studies have been conducted since the 1960s to ascertain the natural products existing in *M. speciosa.* The primary compounds are indole alkaloids, in which approximately 54 alkaloids have been isolated and identified to date (Flores-Bocanegra et al., 2020). Mitragynine is the main indole alkaloid that makes up approximately 66% of the total alkaloidal content of *M. speciosa* (Ponglux et al., 1994; Gogineni et al., 2014). The variations in the percentage of mitragynine is dependent on regional disparities, season and the maturity of plants (Leon et al., 2009; Pearson et al., 2018; Karunakaran et al., 2022). For instance, older plants have a higher percentage of mitragynine than the younger plants (Gogineni et al., 2014). Meanwhile, kratom leaves from Thailand possess a higher mitragynine content at 66%, whereas Malaysian *M. speciosa* specimens is reported to be at 12% (Takayama et al., 1998; Gogineni et al., 2014). However, recent evidence by Goh and colleagues (2021) found that the Malaysian *M. speciosa* variant ranges in mitragynine content from 6.53% to 7.19%.

In addition to mitragynine, several interesting alkaloids are also found in *M. speciosa* which include paynantheine (9%), speciogynine (7%), 7 hydroxymitragynine (2%), and speciociliatine (1%) (Shellard, 1974; Ponglux et al., 1994; Hassan et al., 2013). While mitragynine is purported to be the most prevalent active alkaloid of the plant (Takayama, 2004; Tanguay, 2011; EMCDDA, 2019), 7 hydroxymitragynine has been shown to exhibit potent opioid agonistic properties (Matsumoto et al., 2005a). In fact, 7 hydroxymitragynine was shown to have 46-fold greater potency compared to mitragynine and 13-fold greater potency than morphine (Matsumoto et al., 2004; Adkins et al., 2011). Therefore, these two alkaloids are said to be pharmacologically active along with corynantheidene and speciociliatine

(Chear et al., 2021), although additional alkaloids could also, in an unknown manner, contribute synergistically to the effects (Babu et al., 2008; Singh et al., 2016; Feng et al., 2017; Eastlack et al., 2020).

2.1.6 Pharmacological effects

Comprehensive research on the pharmacological activities of the alkaloids from *M. speciosa* have been undertaken, with an emphasis on the primary indole alkaloid, mitragynine since it is the most abundant constituent of the leaf. Macko and colleagues (1972) were the first to report on the pharmacological properties of mitragynine, where mitragynine was shown to exert its anti-nociceptive activity in rodents (i.e. rats and mice) and dogs when administered orally and intraperitoneally. In addition, an anti-nociceptive study on the methanolic and alkaloid *M. speciosa* extracts demonstrated that the extracts had extended the latency of responses to noxious stimulation in the hot-plate test only and not in the tail-flick test (Reanmongkol et al., 2007). Correspondingly, a study carried out by Mossadeq and colleagues (2009) found that mice given intraperitoneal administration of kratom methanolic extract showed prolonged anti-nociceptive activity in the hot-plate test as well. Further evaluation with acetic-acid-induced writhing and formalin test confirmed that the methanolic extract exhibits anti-nociceptive properties as evident by a significant reduction in writhing responses and pain sensation in both tests (Mossadeq et. al., 2009).

In addition, *M. speciosa* and its derivatives possess anti-inflammatory properties as well. The administration of the methanolic extract of kratom is reportedly capable of impeding the development of carrageenan-induced paw

oedema in rats, which typifies acute inflammation (Mossadeq et al., 2009). In the same study, it was found that 7 consecutive days of methanol extract treatment could suppress the formation of granuloma tissue. The authors postulated that the anti-inflammatory effects may be contributed by several factors which include enhanced immunity, increased vascular permeability, stimulation of tissue repair and healing processes and inhibition of the release of pro-inflammatory mediators (Mossadeq et al., 2009). Another study investigating the cellular mechanism involved in the anti-inflammatory effects of mitragynine revealed that mitragynine was able to hinder the COX-2 mRNA and protein expression as well as PGE_2 (i.e.one of the strongest inflammatory mediators) in RAW264.7 macrophage cells in a dose-dependent manner (Utar et al., 2011).

Moreover, kratom seems to have gastrointestinal effects. For instance, *M. speciosa* extract administered acutely and chronically, demonstrated an anorectic effect when rats showed a reduced food and water intake. Additionally, rats showed a suppressed weight gain (Kumarnsit et al., 2006). It is highly likely that the anorectic properties of kratom was largely contributed by mitragynine which is theorised to be attributed to the inhibitory effects of mitragynine on gastric secretion (Kumarnsit et al., 2006). This result is in accordance with the finding of Jansen and Prast (1988) that kratom users experience anorexia and weight loss. In a separate study, Tsuchiya and colleagues (2002) have revealed that mitragynine dose-dependently inhibited 2-deoxy-D-glucose-stimulated gastric acid secretion in rats.

Moreover, a growing body of research has shown that kratom possesses potential as an anti-psychotic and anti-depressant. A 2016 study determined that the methanolic kratom extract could alleviate apomorphine-induced psychotic symptoms manifested as abnormal cage-climbing behaviour in rats (Vijeepallam et al., 2016).

On the contrary, two methods commonly used in screening of anti-depressant compounds include the forced swimming test (FST) and the tail suspension test. A parameter used in both these studies is immobility time where it refers to the time taken for the animal subject to be completely motionless and immobile (Idayu et al., 2011). A study demonstrated a lowered immobility time in the forced swimming test following the administration of alkaloid-rich fraction of kratom in mice (Kumarnsit et al., 2007) indicating that *M. speciosa* has anti-depressant activity. This finding is supported by Idayu et al. (2011) when mitragynine administered intraperitoneally markedly reduced the duration of immobility in mice that underwent forced swimming test and tail suspension test. In fact, the aforementioned response in immobility time was similar to that of anti-depressant drugs (i.e. amitriptyline, fluoxetine) (Idayu et al., 2011). In the same study, it was also revealed that mitragynine lowered the blood cortisol levels similar to the effects of the anti-depressants in the tests (Idayu et al., 2011). This finding corroborates a clinical research finding on the association between cortisol levels and the possibility of developing major depressive disorders (Jia et al., 2019).

Furthermore, *M. speciosa* has been reported to act as an anxiolytic. Repeated administration of kratom extract (i.e. methanol and aqueous extracts) in rats for 7 consecutive days resulted in an increased time spent in the open arm of the elevated plus-maze indicative of the anxiolytic-like effects of kratom (Moklas et al., 2013). In a separate study, acute oral administration of mitragynine in rats exerted anxiolytic effects in both the open field and elevated plus-maze tests evidenced by an increased time spent in the central zone and open arm respectively (Hazim et al., 2014). Corroborating these findings, acute intraperitoneal administration of mitragynine was

able to exhibit its anxiolytic-like effects in a light/dark box and elevated plus-maze test (Yusoff et al., 2016).

Apart from the above-mentioned pharmacological effects, kratom has been shown to exhibit muscle relaxant properties. According to a study conducted by Chittrakarn et al. (2010), the methanolic extract of kratom and mitragynine could reduce hemidiaphragm muscle twitch contraction in rats. In addition, a study by Matsumoto and colleagues (2005b) revealed that mitragynine could inhibit vas deferens constriction elicited by electrical transmural stimulation. Altogether, this evidence suggests the potential of *M. speciosa* and its derivative, mitragynine, as muscle relaxant. Furthermore, a separate study investigating the anti-bacterial properties of kratom showed that the methanolic extract of kratom had an antimicrobial effect against bacteria causing respiratory tract infection (i.e. *S. pneumoniae*) and bacteria in the digestive tract (i.e. *E. coli*) (Salim et al., 2021).

2.1.7 Toxicity and adverse effects

2.1.7 (a) In humans

Grewal (1932) conducted the first clinical study on mitragynine in humans where he reported mild facial flushing, slight tremors, giddiness, vomiting and nausea. Additionally, multiple studies reported relatively low and mild side effects as well as substance use disorder among traditional Southeast Asian kratom users (Vicknasingam et al., 2010; Singh et al., 2018, 2019b). However, chronic use of high kratom dosage could cause severe adverse effects including agitation, hypertension, palpitations, sleepiness, mouth dryness, tachycardia, nausea, vomiting, anorexia, sweating, psychosis, constipation, itching, darkened skin, confusion and

hallucinatory experiences (Suwanlert, 1975; Jansen & Prast, 1988; Babu et al., 2008; Adkins et al., 2011; Prozialeck et al., 2012, Post et al., 2019). Meanwhile, consuming small doses of kratom (5 g or less) has been shown to cause nausea, itchiness, reduced appetite and high urination frequency (Swogger et al., 2015; Warner et al., 2016). Toxicities were typically reported for kratom doses exceeding 8 g (Eggleston et al., 2019; Jentsch & Pippin, 2022).

Kratom use alone has not been associated with toxicity cases in human as it is relatively rare (Kapp et al., 2011; Prozialeck et al., 2019) but kratom toxicity could cause fatality in some cases. Based on mortality statistics from the Centers for Disease Control and Prevention (CDC), kratom-associated fatalities account for less than 1% of the State Unintentional Drug Overdose Reporting System (SUDORS) overdose deaths from 2016 to 2017 (Olsen et al., 2019). Of the 152 kratom-associated deaths, only 7 were attributed to kratom solely on postmortem toxicology (Olsen et al., 2019). However, it is impossible to dismiss the presence of additional substances in the kratom compound (Olsen et al., 2019; Gershman et al., 2019). For example, certain commercial kratom products which caused unintentional deaths following consumption were found to be adulterated with Odesmethytramadol and phenylethylamine (Arndt et al., 2011; Kronstrand et al., 2011; Nacca et al., 2020). Moreover, the Food and Drug Administration (FDA) discovered the presence of heavy metals in kratom products such as nickel and lead (US Food and Drug Administration, 2021; Prozialeck et al., 2022). Hence, it has become challenging to guarantee the authenticity, potency and purity of kratom products sold commercially without regulatory oversight (Hanna, 2012).

Moreover, poly-drug abuse is one of the biggest contributors to fatality when 87% of kratom toxicity and fatality cases are associated with poly-substance abuse

(Corkery et al., 2019). The co-administration of different drugs (i.e. cocaine, heroin, krypton, opioids, benzodiazepines, fentanyl and alcohol) with kratom could result in addictive or synergistic effects which contributes to accidental death (Kronstrand et al., 2011; McIntyre et al., 2015; Gershman et al., 2019; Davidson et al., 2021). As such, kratom-only case reports are relatively small and extremely rare despite chronic and high dosages of kratom consumption (Ramanathan & Mansor, 2014; Veltri & Grundmann, 2019; Eastlack et al., 2020). It is also important to note that kratom-associated toxicity effects such as hepatotoxicity (Kapp et al., 2011; Dorman et al., 2015; Cinosi et al., 2015; Osborne et al., 2019), seizures (Boyer et al., 2008; Tatum et al., 2018) and death (Kronstrand et al., 2011; McIntyre et al., 2015; Gershman et al., 2019) are mainly documented in Western countries, with a small number of cases reported in Southeast Asia (Veltri & Grundmann, 2019; Prozialeck et al., 2019) which may be due to the processed kratom products. Thus far, there has been no fatalities associated with kratom consumption on its own in Southeast Asia. In spite of the reported toxicity and side effects of kratom, kratom holds a great potential for therapeutic use given the experience of Southeast Asian users (Singh et al., 2016).

2.1.7(b) In animals

The initial animal toxicity study was recorded as early as 1972 when Macko and colleagues (1972) revealed no indication of toxicity, as determined by convulsions or tremors, following oral administration of mitragynine up to 920 mg/kg in mice. However, a previous toxicity study by Janchawee et al. (2007) displayed fatal outcomes in rats administered oral mitragynine at 200 mg/kg. This