STUDIES OF MITRAGYNINE, THE PRINCIPLE ALKALOID OF *MITRAGYNA SPECIOSA* KORTH (KRATOM) ON ITS ABUSE AND ADDICTIVE PROPERTIES IN RAT BEHAVIOURAL MODELS

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by

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LIST OF ABBREVIATIONS

i.p.	Intraperitoneal
i.c.v.	Intracerebroventricular
mRNA	Messenger Ribonucleic Acid
PCR	Polymerase Chain Reaction
i.e.	That is
e.g.	Example
etc.	Et cetera (and other things)
C_{max}	Maximal reaction concentration
T_{max}	Maximal reaction time
LD_{50}	Median lethal dose
IC_{50}	Half maximal inhibitory concentration
ED_{50}	Effective dose at 50% of activity
95% CI	95% of confidence interval
SEM	Standard error mean
ANOVA	Analysis of variance
PVC	Polyvinyl chloride
NaCI	Sodium chloride

µg	Microgram
mg	Milligram
g	Gram
ml	Millilitre
mm	Millimetre
cm	Centimetre
min	Minute (s)
h	Hour (s)
sec	Sec (s)
µg/ml	Microgram per millilitre
mg/ml	Milligram per millilitre
ml/kg	Millilitre per kilogram
mg/kg	Milligram per kilogram
ng/ml	Nanogram per millilitre
v/v	Volume over volume
IU/ml	International unit per millilitre
USA	United States of America
rpm	Revolutions per minute

LIST OF SYMBOLS

%	Percentage
µ	Mu
δ	Delta
κ	Kappa
α	Alpha
±	Plus minus
º	Degree
ºC	Degree Celsius
n	Number of animals
=	Equal to
>	Greater than
≥	Greater than or equal to
≤	Less than or equal to
™	Trade mark
©	Copyright
®	Registered

LIST OF APPENDICES

KAJIAN TENTANG MITRAGININA, ALKALOID UTAMA DARIPADA *MITRAGYNA SPECIOSA* KORTH (KRATOM) KE ATAS SIFAT-SIFAT PENYALAHGUNAAN DAN KETAGIHAN DALAM MODEL-MODEL TINGKAH LAKU TIKUS

ABSTRAK

Mitraginina adalah alkaloid aktif utama yang dipencil daripada daun *Mitragyna speciosa* atau kratom. Sementara mitraginina atau kratom menghasilkan kesan-kesan analgesik, anti-radang dan relaksi otot, namun sedikit yang telah diketahui tentang sifat-sifat penyalahgunaan dan penagihannya. Diberikan kemungkinan kepentingan sistem opioid dalam perantaraan kesan-kesan farmakologi mitraginina, kajian ini bertujuan meninjau kesan-kesan tingkah laku daripada mitraginina menggunakan tatacara pendiskriminasian drug dan pemberian sendiri drug. Tikus jantan jenis Sprague Dawley telah dilatih untuk mendiskriminasi antara mitraginina dan pembawa menggunakan tatacara pendiskriminasian drug dua tuil di bawah pembolehubah selang secara tandem (VI-60 saat) dan nisbah tetap (FR-10) dalam jadual pengukuhan makanan. Tikus-tikus itu telah berjaya memperoleh pendiskriminasian mitraginina (15.0 mg/kg, i.p.) yang mirip dengan kumpulan tikus yang satu lagi dengan pendiskriminasian morfina (5.0 mg/kg, i.p.) Untuk memperoleh pemahaman yang lebih baik tentang penyerupaan farmakologi yang dikongsi antara mitraginina dan morfina, siri-siri ujian penukargantian yang serupa telah dijalankan pada tikus yang dilatih untuk mendiskriminasi morfina daripada pembawa. Mitraginina telah ditukargantikan sepenuhnya kepada rangsangan diskriminatif morfina dan sebaliknya, mencadangkan penyerupaan farmakologi di

antara kedua-dua jenis drug. Mekanisme farmakologi daripada derivatif mitraginina, 7-hidroksimitraginina dengan fokus yang spesifik kepada penglibatan reseptor opioid juga telah diperiksa pada tikus-tikus yang dilatih untuk mendiskriminasi morfina daripada pembawa. Pemberian derivatif 7-hidroksimitraginina pada dos 3.0 mg/kg (i.p.) mengakibatkan penukargantian penuh kepada rangsangan diskriminatif morfina. Dalam usaha mengkaji kesan tindakan dual mitraginina, kesan penukargantian kokaina terhadap rangsangan diskriminatif mitraginina telah dijalankan pada tikus-tikus yang telah dilatih dengan mitraginina. Rangsangan mitraginina menunjukkan penukargantian separa ke atas rangsangan kokaina (10.0 mg/kg, i.p.) manakala kesan ini tidak dapat dilihat dalam tikus-tikus yang dilatih untuk mendiskriminasi morfina daripada pembawa. Oleh kerana sifat-sifat subjektif yang menyerupai opioid dikesan melalui tatacara pendiskriminasian drug, kajian selanjutnya bertujuan untuk menilai profil mitraginina dalam model tikus yang menunjukkan kebergantungan kepada fentanil (agonis μ-opioid) menggunakan tatacara dua tuil 'pilihan' pemberian sendiri drug. Mitraginina dalam tiga unit dos (0.3, 1.0 dan 3.0 mg/kg/infusi) telah mengekalkan gerak balas tekan tuil daripada pemberian sendiri fentanil (2.0 μg/kg/infusi). Profil sepanjang ujian saling penukargantian mitraginina berbeza daripada aras-aras garis dasar yang diperlihatkan dalam ujian-ujian pemupusan, lalu mencadangkan mitraginina mungkin mempunyai kesan-kesan pengukuhan. Keseluruhannya, kesan rangsangan diskriminatif dan potensi penghasilan kebergantungan yang ditunjukkan oleh mitraginina memberikan sebahagian bukti kepada potensi penyalahgunaan dan ketagihannya yang boleh menyokong pengelasan mitraginina atau kratom sebagai dadah yang berbahaya.

STUDIES OF MITRAGYNINE, THE PRINCIPLE ALKALOID OF *MITRAGYNA SPECIOSA* KORTH (KRATOM) ON ITS ABUSE AND ADDICTIVE PROPERTIES IN RAT BEHAVIOURAL MODELS

ABSTRACT

Mitragynine is the main active alkaloid isolated from the leaves of *Mitragyna speciosa* or kratom. While mitragynine or kratom provides analgesic, anti-inflammatory and muscle relaxant effects, little is known about its abuse and addictive properties. Given the likely importance of opioid system in mediating the pharmacological effects of mitragynine, the present study aims to explore the behavioural effects of mitragynine using drug discrimination and drug self-administration procedures. Male Sprague Dawley rats were trained to discriminate between mitragynine and vehicle in two-lever drug discrimination procedure under a tandem variable-interval (VI-60 sec) and fixed-ratio (FR-10) schedule of food reinforcement. The rats successfully acquired mitragynine discrimination (15.0 mg/kg, i.p.) which was similar to another group of rats with morphine discrimination (5.0 mg/kg, i.p.). To gain a better understanding on the pharmacological similarities shared between mitragynine and morphine, a similar series of substitution tests were conducted in rats trained to discriminate morphine from vehicle. Mitragynine was fully substituted to the morphine discriminative stimulus and vice versa, suggesting pharmacological similarities between the two drugs. The pharmacological mechanism of mitragynine derivative, 7-hydroxymitragynine with a specific focus on opioid receptor involvement was also examined in rats trained to discriminate morphine from vehicle. The administration of 7-hydroxymitragynine derivative at

3.0 mg/kg (i.p.) dose engendered full substitution to the morphine discriminative stimulus. In order to study the dual actions of mitragynine, the effect of cocaine substitution to the mitragynine discriminative stimulus was performed in mitragynine-trained rats. The mitragynine stimulus was partially substituted to cocaine (10.0 mg/kg, i.p.) stimulus while this effect was not observed in rats trained to discriminate morphine from vehicle. Due to the 'opioid-like' subjective properties detected in drug discrimination model, further studies assessed the profile of mitragynine in rat model of fentanyl (*i.e.* μ-opioid agonist) dependence using two-lever 'choice' of self-administration procedure. Mitragynine at three unit doses (0.3, 1.0 and 3.0 mg/kg/infusion) maintained the lever-pressing responses of the fentanyl (2.0 μg/kg/infusion) self-administration. The profile over the mitragynine cross-substitution tests was different from baseline levels observed during extinction tests, suggesting that mitragynine may have its reinforcing effects. Altogether, the discriminative stimulus effect and dependence-producing potential exhibited by mitragynine provide some evidence for its abuse and addictive potential, which may support the classification of mitragynine or kratom as a harmful drug.

CHAPTER 1

INTRODUCTION

It has been documented that natural products have contributed to development of new psychoactive drugs primarily due to pain relieving effects. Recently, new natural products appear with more than doubled over the period of 2009 to 2013 on the drug markets (UNODC, 2014). The emerging search of new psychoactive drugs brings forward the long traditional use of the products to the global markets (Rosenbaum et al., 2012). Regardless of the efficacy of the products which are yet to be proven in controlled clinical trials, such products are being used extensively. Although they are considered safe due to their natural origins (Kronstrand et al., 2011), animal studies and clinical reports such as toxicity, adverse effects, as well as abuse-and addictive-related effects may indicate their potential risks to humans.

One natural product that appears on the drug scene and gains widespread popularity in Malaysia is *Mitragyna speciosa* Korth or kratom (Adkins et al., 2011; Hassan et al., 2013). Mitragynine is the main active alkaloid extracted from the leaves of *M. speciosa* Korth (Shellard, 1974; Ponglux et al., 1994). For this reason, mitragynine is assumed to be the major chemical responsible for the effects of the plant. There are also other constituents of *M. speciosa* that may contribute to the effects which may involve 7-hydroxymitragynine, a minor constituent of the leaves extracts (Ponglux et al., 1994). Many scientific reports have indicated that mitragynine produces a variety of pharmacologic effects which include the induction

of analgesic effects (Matsumoto et al., 1996a; Stolt et al., 2014), inhibition of ileum motility (Watanabe et al., 1997) and vas deferens contractions of smooth muscle (Matsumoto et al., 2005b), as well as the inhibition of gastric acid secretion (Tsuchiya et al., 2002), which is consistent with the actions of morphine. In addition, studies with opioid receptor antagonists indicate that the effects are primarily mediated by the actions on opioid receptors (Matsumoto et al., 2005b; Watanabe et al., 1997).

However, the psychoactive properties of *M. speciosa* appear to be contradictory as there are reports claiming it possesses both narcotic and stimulant properties (Assanangkornchai et al., 2007; Macko et al., 1972; Ward et al., 2011; Grewal, 1932a; Grewal, 1932b; Suwanlert, 1975, Reanmongkol et al., 2007). This suggests that kratom produces an unusual combination of opioid- and psychostimulant-like effects. Nevertheless, the pharmacological basis underlying the dual effects of mitragynine is still unclear. The stimulant and euphoric effects exerted by kratom caused the plant to be extensively misused as a herbal drug. Due to easy availability and cheap herbal preparation, kratom abuse has gained significant attention in Malaysia and Thailand (Chan et al., 2005). Furthermore, the wide availability of kratom to purchase through the internet reflects an increasing demand of misusing this herbal product globally (Boyer et al., 2008).

The likelihood that a drug will be abused is generally based on the subjective and reinforcing effects exerted by the drug (Moser et al., 2011b). This has provided the framework for animal models of drug of abuse. Drug discrimination is used as a model to study the subjective effects of psychoactive drugs since the action of a drug's discriminative stimulus in animals is closely related to its subjective effects in

human (Overton, 1988). Given that the action of mitragynine resembles morphine-like (opioid) effects, it was hypothesised that mitragynine may share discriminative stimulus properties with morphine. Intravenous drug self-administration model is used to assess the reinforcing effects of drugs to reveal the reasons why humans abuse and become addicted to drugs (Shoaib, 2006). A cross-substitution experiment in which rats previously trained to self-administer intravenous injections of opioid will be presented with various doses of mitragynine. The dependence-producing potential of mitragynine from rat model of opioid dependence provides information on its potential reinforcing effects.

The research to date has tended to focus on the effects of kratom on health, psychological, cognitive, behavioural and social impact (Macko et al., 1972; Moklas et al., 2008; Apryani et al., 2010; Idayu et al., 2011; Ahmad and Aziz, 2012; Sabetghadam et al., 2013a; Sabetghadam et al., 2013b; Yusoff et al., 2014) with little attention towards the assessment of abuse and addictive potential of the plant extracts or its active compounds. The present study addresses the assessment of abuse and addictive properties of the naturally occurring compound mitragynine, the major psychoactive constituent of *M. speciosa* Korth or kratom using rat behavioural models.

1.1 Problem statements

Kratom has been widely used for decades for both medical and non-medical purposes. Even though the efficacies are yet to be scientifically proven in humans, kratom is continued to be used extensively. The available human reports on kratom abuse and addictive properties have heightened the need for the assessment of its abuse and addictive potential.

As a major constituent of *M. speciosa*, mitragynine is known as a psychoactive compound that produces major pharmacologic effects at opioid receptors. Most studies in the past decades have shown the resemblance of mitragynine towards morphine-like pharmacological characteristics. Thus, there is an increasing concern that mitragynine is being disadvantaged to the similar extent as the established opioid (*i.e.* morphine). Since drugs of abuse in humans produce subjective effects in experimental animals, there is a paucity of research whether mitragynine produces its subjective effects in rat behavioural models.

In addition, dual properties of opium-like (opioid) and coca-like (psychostimulant) effects demonstrated by mitragynine and kratom extracts represent the complexity in understanding their mechanisms of action. This situation raises an interest in elucidating the pharmacological similarity between mitragynine and opioid (*i.e.* morphine) and between mitragynine and psychostimulant (*i.e.* cocaine).

Besides mitragynine, 7-hydroxymitragynine also becomes another alkaloid of interest when it is reported as a minor psychoactive compound present in the plant. 7-Hydroxymitragynine has been thought as another key component that contributes

towards the opium-like properties. Thus, it is suggested that the compound may also associate with the opioid discriminative stimulus effects.

In addition, drug abuse and addiction are often characterised by their reinforcing effects. The elucidation of mitragynine dependence-producing potential in opioid-dependent animals is warranted to provide information on its reinforcing effect.

Last but not least, striking increases in the number of opioid users have expanded the need for pharmacotherapeutic interventions. Even though human reports and pre-clinical studies suggested potential valuable therapeutic properties of kratom, the neurobiological mechanism of using kratom as a substitution treatment amongst opioid addicts is still unclear. In fact, there is lack of scientific data to support the use of kratom as a potential substitution treatment.

1.2 Objectives of study

Six specific objectives are constructed in this study in order to address the abovementioned problem statements:

(i) To demonstrate the induction of mitragynine discrimination in rats

(ii) To evaluate the substitutability of mitragynine to morphine and cocaine

(iii) To evaluate the substitutability of morphine to mitragynine and cocaine

(iv) To evaluate the effect of 7-hydroxymitragynine derivative on discriminative stimulus effect of morphine

(v) To investigate the effect of naloxone, an opioid antagonist on discriminative stimulus effect of mitragynine, morphine and 7-hydroxymitragynine substituted responses

(vi) To assess the dependence profile of mitragynine in a rat model of fentanyl self-administration

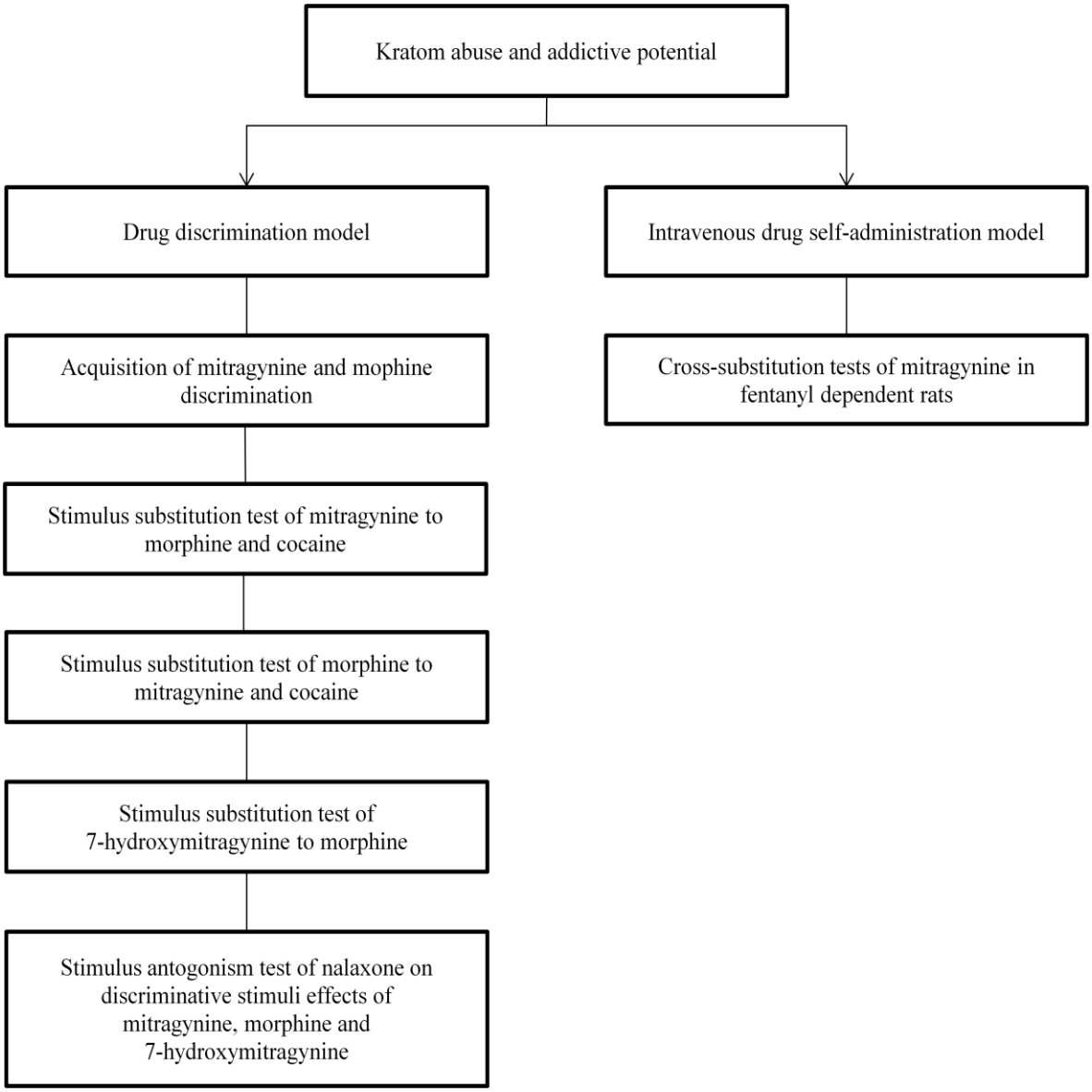


Figure 1.1: The experimental design demonstrating the assessment of kratom abuse and addictive potential using two behavioural models

CHAPTER 2

LITERATURE REVIEW

2.1 *Mitragyna speciosa* Korth (kratom)

Mitragyna speciosa Korth is a tropical plant indigenous to Southeast Asia region particularly in Peninsular of Malaysia and Thailand (Jansen and Prast 1988a; Hassan et al., 2013). The plant is known as 'biak-biak' or 'ketum' in Malaysia and as 'kratom' in Thailand (Jansen and Prast, 1988a; Adkins et al., 2011). It has been used as herbal stimulant by labourers and farmers in increasing work productivity during harsh conditions (Grewal, 1932a; Suwanlert, 1975; Hassan et al., 2013). The plant is known for decades as traditional herbal medicine for common illnesses such as diarrhoea, coughing, fever, malaria, muscle pain, as well as to improve blood circulation, sexual performance and to treat diabetes (Watanabe et al., 1997; Chan et al., 2005; Assanangkornchai et al., 2007; Kumarnsit et al., 2007a ; Chittrakarn et al., 2010; Hassan et al., 2013).

Apart from the energising and medicinal properties, kratom has been used as a substitute for opiate addiction due to the capability of suppressing opioid withdrawal symptoms (Wray, 1907; Suwanlert, 1975; Vicknasingam et al., 2010; Ahmad and Aziz, 2012). The earliest use of kratom substitution for opium was reported in Malaysia as early as 1895 (Wray, 1907; Burkill, 1935).

2.1.1 Botanical origin

Mitragyna speciosa Korth, as shown in Figure 2.1 belongs to the *Rubiaceae* family. The plants grow freely in swampy areas, fertile soil with sufficient sun exposure in regions that are protected from the strong winds (Shellard and Lees, 1965; Macko et al., 1972; Hassan et al., 2013). They are large trunk trees which can grow up 4 to 9 metres height and 5 metres wide. The dark yellow flowers are globular in shape bearing up to 120 florets attached to the leaf axils on long stalks. The dark glossy green colour leaves normally grow to over 18 cm long and 10 cm wide with an ovate acuminate shape and tapered ends (Shellard and Lees, 1965; Hassan et al., 2013). The leaves will normally fall abundantly during hot season and grow intensely during rainy season (Macko et al., 1972). From all parts of the plant, the leaves are generally consumed (Jansen and Prast, 1988b; Kumarnsit et al., 2006; Hassan et al., 2013; Saingam et al., 2013) for their effects.

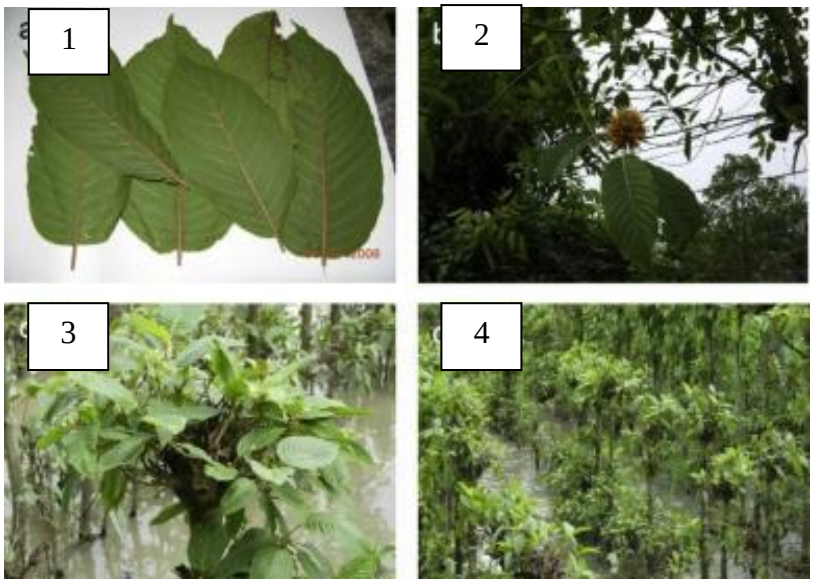


Figure 2.1: The plant of *Mitragyna speciosa* Korth (1) Leaves of the plant, (2) naturally occurring trees, (3) and (4) cultivated plants. Pictures are adopted from Hassan et al., (2013).

2.1.2 Plant preparation and consumption

There are various ways to prepare kratom leaves. Some users simply chew the fresh leaves (Wray, 1907; Jansen and Prast, 1988a; Hassan et al., 2013) while some prefer kratom in the form of dried powder, in which they can smoke, swallow or prepare as tea for ingestion (Wray, 1907; Jansen and Prast, 1988a; Babu et al., 2008). Locals will usually dry the leaves when kratom is abundance and use it as tea when there is a shortage of supply. The kratom users claim to experience no difference between the effects of fresh and dried leaves (Saingam et al., 2013; Sakaran et al., 2014). To make it easier for consumption, kratom is sold as drinks with a cheap price. In Malaysia, a small packet of the drink is sold at RM 1 (RM 1 = 0.30 USD) (Chan et al., 2005; Ramanathan and Mansor, 2014). Sometimes, the drinks are consumed together with sugar or honey to mask their bitter taste. Lemon juice and salt are also added to facilitate the extraction of kratom leaves and prevent

constipation respectively (Jansen and Prast, 1988a; Tanguay, 2011; Hassan et al., 2013). In Southern Thailand, kratom is sometimes mixed with caffeine-containing soft drinks and codeine- or diphenhydramine-containing cough syrup to give the anxiolytic, antidepressant and analgesic effects (Tanguay, 2011; Hassan et al., 2013). Recently, kratom drink is mixed with some other toxics or addictive substances (*i.e.* cough medication, rat poison and mosquito coils) to increase the euphoric effects and give extra flavours to the drink (Saingam et al., 2013; Ramanathan and Mansor, 2014).

Nevertheless, many users tend to believe that kratom is not as harmful as other illicit drugs like heroin, morphine, cocaine, methamphetamine and etc. This may explain the increasing demand for kratom which not only observed in Malaysia and Southeast Asia countries but also in other parts of the world. In recent years, kratom has spread to Europe and United States where its products are readily available via internet, ranging from dried powder leaves to capsules, tablets and concentrated extracts (Boyer et al., 2007; Boyer and Wines, 2008). Kratom is also popular as a herbal blend krypton when it is mixed with O-desmethyltramadol, an active metabolite of a commonly prescribed analgesic (*i.e.* tramadol) to give more powerful effects (Backstrom et al., 2010; Kronstrand et al., 2011). The easy availability and cheap price of kratom compared to other illicit drugs reflect the wide spread of misusing the natural product (Boyer et al., 2007; McWhirter and Morris, 2010; Adkins et al., 2011; Ramanathan and Mansor, 2014). The pictures of kratom consumption and several kratom products are shown in Figure 2.2A and Figure21.2B respectively.

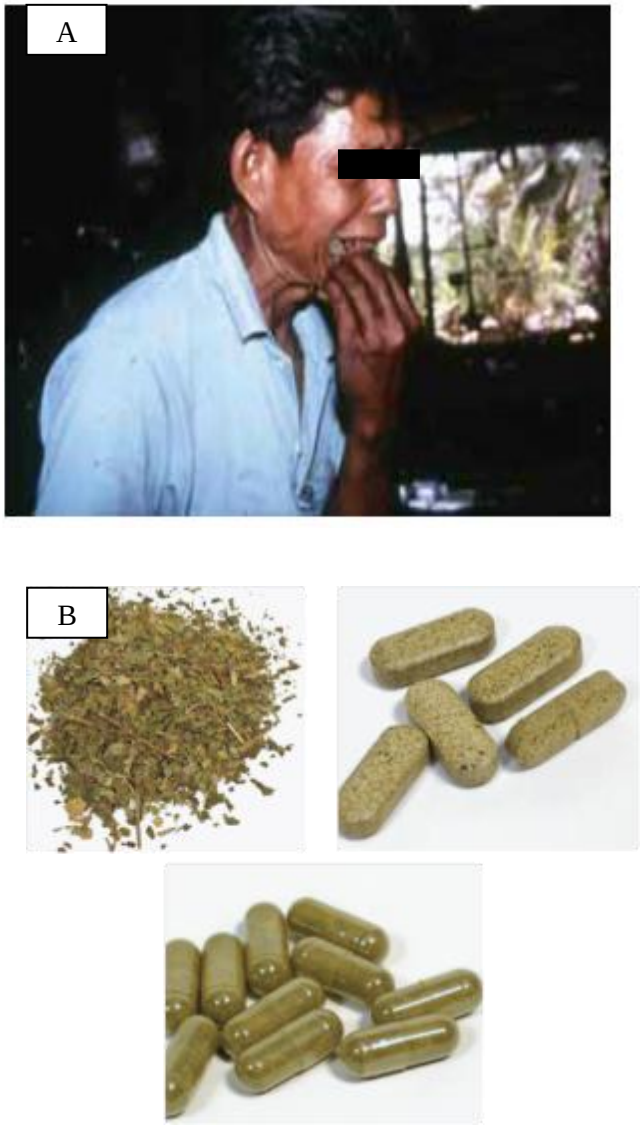


Figure 2.2: (A) A kratom user chewing the kratom leaves. Picture is adapted from Matsumoto, (2006) (B) Products of kratom sold in the market. Pictures are adopted from Prozialeck et al. (2012).

2.1.3 Kratom consumption in humans

Typically, kratom is popular among elderly who find it beneficial for enhancing their physical energy and increasing work efficiency (Suwanlert, 1975; Vicknasingam et al., 2010; Saingam et al., 2013). Thus, people who consume kratom are regarded as better than those who abuse illicit drugs because they will become energetic while illicit drugs users usually feel lazy (Saingam et al., 2013). Besides, kratom users believe that their kratom practice is harmless and socially accepted as they do not disturb anyone which is opposite to the effects of illicit drugs (Assanangkornchai et al., 2007; Saingam et al., 2013). In fact, 99% of the kratom users still take a good care of their families (Ahmad and Aziz, 2012).

In addition, kratom is also used as traditional herbal medication for various health disorders such as diabetes, hypertension, coughing, diarrhoea and muscle pain which enabling them for self-treatment (Suwanlert, 1975; Saingam et al., 2013). The knowledge of kratom as herbal medication is passed from generation to generation, keeping such treatment available to be practised (Saingam et al., 2013). Interestingly, the beneficial use of kratom is supported by recent pharmacological studies when kratom and its active constituents have demonstrated analgesic, antidiabetic and antidiarrheal effects, the morphine-like action on gastric acid secretion and reduction of morphine withdrawal symptoms in animal studies (Matsumoto et al., 1996b; Tsuchiya et al., 2002; Kumarnsit et al., 2007a; Reanmongkol et al., 2007; Khor et al., 2011).

The Northern states of Malaysia (*i.e.* Kedah and Perlis), which are located at the border of Thailand have become a ‘redspot’ for kratom (Chan et al., 2005; Singh

et al., 2014). This leads to a cross-sectional survey done by Singh et al. (2014) to reveal various reasons of kratom use and the result is shown in Table 2.1. Majority of the respondents with 28% consumed kratom to increase their physical energy. There are about 15% of the respondents who consumed kratom to abstain from the effects of illicit drugs. Meanwhile, 13% of them consumed kratom as self-treatment for their medical problems. A small percentage (*i.e.* 6%) of the respondent claimed that kratom improved their mood, making them happy and relax. This finding coincides with previous reports when kratom users described the effects of 'well-being' or euphoria induction following kratom consumption (Jansen and Prast, 1988a; Jansen and Prast, 1988b; Assanangkornchai et al., 2007). Similar reasons of using kratom amongst the users were also reported in Thailand (Assanangkornchai et al., 2007; Saingam et al., 2013).

Despite of its effects, about 21% of the respondents reported that they were using kratom due to curiosity and peer influence. This survey aligns with Thailand study when kratom users reported their initial use was due to curiosity and peer-pressure (Assanangkornchai et al., 2007). This finding is supported by Saingam et al. (2013) where kratom is known amongst young people for fun or relaxation purposes.

Table 2.1: Reasons for using kratom in specific population at Northern Malaysia

	Respondent (n)	(%)
To enhance physical energy	83	28
Curiosity	61	21
Peer influence	46	16
To abstain from illicit drugs/alcohol	45	15
Self-treatment	38	13
To improve mood/ease boredom	17	6
To relieve fatigue	3	1
Total	293	100

* Adopted from Singh et al. (2014)

2.1.4 Phytochemistry

The chemistry of *M. speciosa* has been continuously studied for years. The investigation began in the 1920s when Hooper and Field first reported the isolation of mitragynine compound from *M. speciosa* leaves extracts (Field, 1921; Jansen and Prast, 1988a). However, the chemical structure of mitragynine was fully determined only almost 30 years later by Zacharias et al. (1965). A study by Shellard et al. (1974) later resulted in the isolation of over 25 alkaloids from various samples of *M. speciosa* leaves extracts. The main alkaloids present in the leaves of *M. speciosa* are mitragynine (12 to 66%) and its analogues that include paynantheine (9%), speciogynine (7%) and speciociliatine (1%) (Shellard, 1974; Hassan et al., 2013). A new analogue, 7-hydroxymitragynine was subsequently isolated as a minor

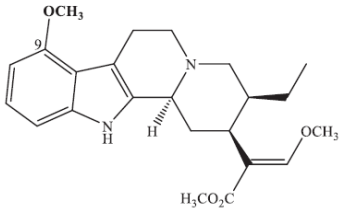
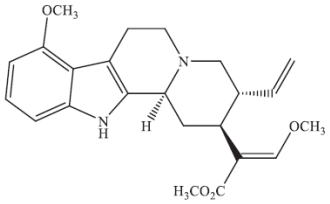
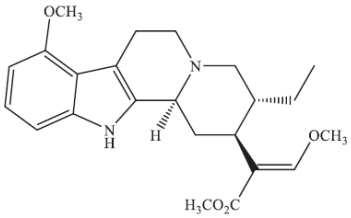
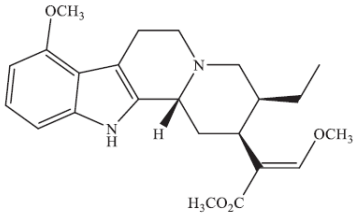
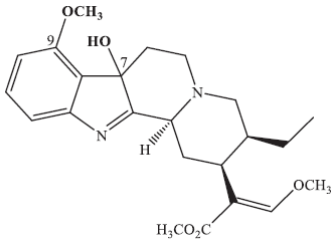
constituent (2%) (Ponglux et al., 1994). Kratom leaves contain approximately 0.2% mitragynine by weight of the total kratom leaves (Grewal, 1932a; Suwanlert, 1975). However, the mitragynine content was reported to vary by geographical region and season (Shellard, 1974; Hassan et al., 2013) as report shows that higher percentage of mitragynine (66%) of total alkaloid extracts of *M. speciosa* leaves was extracted from the leaves produced in Thailand compared to only 12% from the leaves extracted in Malaysia (Takayama, 2004).

As the major constituent, mitragynine is assumed to be mostly responsible for the pharmacological effects of the plant extracts and this has led to an increased interest in characterising its chemical, toxicological and pharmacological properties in recent years (Macko et al., 1972; Shellard, 1974; Matsumoto et al., 1996a; Takayama et al., 2002; Takayama, 2004; Matsumoto et al., 2005b; Reanmongkol et al., 2007; Azizi et al., 2010; Taufik Hidayat et al., 2010; Sabetghadam et al., 2013a). Structurally, mitragynine or 9-methoxy-corynantheidine ($C_{23}H_{30}N_20_4$) with a molecular weight of 398.5 g/mol (Kumarnsit et al., 2007b) consists of a methoxy group at C19 position and C9 position of the indole ring (Beckett et al., 1966a; Beckett et al., 1966b; Shellard, 1974).

Previous studies have reported that the antinociceptive effect of pure mitragynine was less potent than that of the crude extract of the plant (Watanabe et al., 1997; Yamamoto et al., 1999; Matsumoto et al., 2006) which suggested the presence of other active compounds in the total alkaloid extracts. This may involve 7-hydroxymitragynine, the minor constituent of the leaves extracts. 7-Hydroxymitragynine is the compound derived from mitragynine with the introduction of a hydroxyl group at the C7 position of the mitragynine skeleton

(Ponglux et al., 1994). This claim is supported by evidence that the antinociceptive effect of 7-hydroxymitragynine is relatively more potent than mitragynine (Matsumoto et al., 2004; Takayama, 2004). Thus, mitragynine and 7-hydroxymitragynine are reported to be the most pharmacologically active, although the other constituents may also possess the pharmacological effects as listed in Table 2.2.

Table 2.2: Structures and potential effects of alkaloids extracted from kratom

Compound	Percentage	Structures	Structures and pharmacological activities
Mitragynine	12-66%		Structurally similar to yohimbine Analgesic, antitussive, anti-diarrheal, adrenergic, antimalarial effects Activity on μ, δ and κ receptors Activates descending noradrenergic and serotonergic pathways in spinal cord
Paynantheine	9%		Smooth muscle relaxant Inhibits twitch contraction without naloxone blocking effect Inhibits muscarinic receptors on smooth muscle
Speciogynine	7%		Smooth muscle relaxant Inhibits twitch contraction without naloxone blocking effect Inhibits muscarinic receptors on smooth muscle
Speciociliatine	1%		C3 stereoisomer of mitragynine Possess other than stimulation on opioid receptors *i.e.* may inhibit acetylcholine release from presynaptic nerve
7-hydroxy mitragynine	2%		Possess –OH group on C7 position Analgesic, antitussive, anti-diarrheal effects Higher opioid agonist potency than mitragynine and morphine

Note: Percentage denotes the percentages (%) of the compound in the alkaloid fraction/extract

*Adopted from Hassan et al. (2013)

2.1.5 Pharmacokinetics

The quantification analyses of kratom alkaloids in the biological systems are fundamental in order to provide better understanding of the pharmacological activity of its extracts. The pharmacokinetic of mitragynine was well characterized in rat plasma following oral and intravenous administrations. Oral administration of 40 mg/kg of mitragynine in rats led to a peak plasma concentration (C_{max}) of 0.63 μg/ml after T_{max} of 1.83 h (Janchawee et al., 2007) using a High Performance Liquid Chromatography-Ultra Violet (HPLC/UV) method. In the following study using HPLC and tandem mass spectrometry (LC-MS/MS), mitragynine was reported to be rapidly absorbed with a maximum concentration (C_{max}) of 423.68 ng/ml and a T_{max} of 1.26 h after oral administration of 20 mg/kg mitragynine. The elimination half-life ($t_{1/2}$) was shown to be 3.85 h (de Moraes et al., 2009). A pharmacokinetic study done by Parthasarathy et al. (2010) using solid-phase extraction and rapid HPLC-UV analysis reported that oral administration of 50 mg/kg dose of mitragynine resulted in the C_{max} of 0.7±0.21 μg/ml and the T_{max} of 4.5 ± 3.6 h. The elimination half-life ($t_{1/2}$) was 2.9 ± 2.1 h. The variations of the pharmacokinetic profiles observed from these studies could be attributed by poor oral absorption of mitragynine.

Meanwhile, following intravenous administration of 1.5 mg/kg, the C_{max} was reported to be 2.3 ± 1.2 μg/ml with T_{max} of 1.2 ± 1.1 h and elimination half-life ($t_{1/2}$) was determined at 2.9 ± 2.1 h (Parthasarathy et al., 2010). In contrast, a more recent pharmacokinetic study using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) reported the C_{max} of 3.9 ± 0.7 μg/ml at 1 min following the administration of 5 mg/kg mitragynine while the elimination half-life ($t_{1/2}$) was 2.6 ±

0.6 h (Vuppala et al., 2011). However, it is important to note that the pharmacokinetic on multiple dose study of mitragynine has yet to be reported.

2.1.6 Toxicity studies in animals

The earliest acute toxicity report of mitragynine by Macko et al. (1972) found no absolute evidence of toxicity, whereby no tremors or convulsions were observed at the doses as high as 920 mg/kg in mice. However, a recent study by Janchawee et al. (2007) demonstrated lethal effects after an oral administration of 200 mg/kg mitragynine in rats. The similar fatal effect was also observed after administration of 200 mg/kg alkaloid extract of *M. speciosa* to rats (Azizi et al., 2010).

The earliest sub-acute toxicity study by Macko et al. (1972) demonstrated no side effects in rats after administration of 5 or 50 mg/kg/day of mitragynine for five days per week within six weeks duration. There was only a significant decrease in liver and kidney weights that were observed at higher mitragynine dose. Similarly in dogs, no side effects were observed following 20 mg/kg of mitragynine administration per day for three weeks duration. However, hematological findings including leukopenia, lymphocytosis, granulocytopenia, monocytosis and immature lymphocytes were observed in dogs from day 22 when the dose was increased to 40 mg/kg/day for six days per week. In fact, these changes were reversed after the drug was withdrawn. A most recent sub-chronic study by Sabetghadam et al. (2013b) demonstrated no mortality or toxic effects in mice after administered at low or intermediate dose (*i.e.* 1 and 10 mg/kg) of mitragynine. However, decreases in food intake and body weight were observed at the highest mitragynine dose (*i.e.* 100

mg/kg). Overall, the previous reports suggested that mitragynine is relatively safe at low sub-chronic doses (*i.e.* 1 to 10 mg/kg), but exhibits toxicity at higher sub-chronic dose (*i.e.* 100 mg/kg at 28 days) (Sabetghadam et al., 2013b). Nevertheless, information about the dose level that could be translated from animal data to human perspectives is still lacking.

2.1.7 Toxicity and adverse effects in humans

Several human studies and case reports also emerged to provide evidence of long term toxicity and adverse effects of kratom preparations. The first study of mitragynine in humans was reported by Grewal (1932b) when some of the human subjects developed nausea, vomiting, slide tremors of the hand and tongue, giddiness, feeling of haziness and slight flushing of the face following mitragynine administration. It was reported that a man who tried to abstain from kratom had difficulty to sleep, wriggling sensation in the shoulders and the back, dragging sensation in the hips, bitemporal headache, became extremely weak and also could hardly walk (Thuan, 1957). A recent study by Vicknasingam and colleagues (2010) also revealed that kratom produced mild side effects such as loss of weight, dehydration, constipation but no other medical problems were reported. However, prolonged use of kratom was reported to cause adverse effects which include nausea, diarrhoea, vomiting, hallucinations, psychosis, agitation, dizziness, itching, sweating, dry mouth, respiratory depression, constipation, anorexia, increased urination, palpitations and weight loss (Suwanlert, 1975; Jansen and Prast, 1988a; Babu et al., 2008; Adkins et al., 2011; Prozialeck et al., 2012).

Other than adverse effects, there are no reports of mortalities following mitragynine or kratom consumption alone, even after chronic and high dosage consumption (Ramanathan and Mansor, 2014). However, the combination of kratom with other drugs (*i.e.* krypton) (Backstrom et al., 2010; Hassan et al., 2013) had contributed to unintended death (Kronstrand et al., 2011). A similar fatal case was also reported following the co-ingestion of propylhexedrine and mitragynine (Holler et al., 2011). Even though the contribution of kratom or its active compound, mitragynine to the unintended deaths remain unclear, the adulterated form of kratom products could contribute to its multidrug actions. To date, little is known about the clinical effects and the potential toxicities of mitragynine or kratom in humans. The available reports in humans may suggest the potential adverse effects at extremely high dosage of mitragynine or kratom consumption. However, there are no precise studies on mitragynine or kratom dose ranges that are responsible for those effects at present.

2.1.8 Metabolism

Mitragynine is extensively metabolised in rats and humans. This mechanism involved the hydrolysis of the methylester in position C16, O-demethylation of the 9-methoxy group and of the 17-methoxy group followed by oxidation and reduction reactions from the intermediate aldehydes to form carboxylic acids and alcohol, respectively. Finally, four phase 1 metabolites were conjugated to form four glucuronides and one sulphate in rats while the conjugation resulted in three glucoronides and three sulphates in humans (Philipp et al., 2009). Previous report has demonstrated that *M. speciosa* extract showed the most potent inhibition on

cytochrome P450 enzyme (*i.e.* CYP 2D6) with IC_{50} value of 3.6 ± 0.1 µg/ml suggesting that the consumption of the extract together with other drugs that have the same metabolic pathway may contribute to herb-drug interactions (Hanapi et al., 2010). This finding possibly suggests that the mixture of kratom preparations with other psychoactive drugs may lead to overdose and adverse effects.

2.1.9 Opioid agonist property

Opioid is any chemicals that possessed opiate-like pharmacological effects. An opiate is a plant-derived material product from opium poppy (*Papaver somniferum*) (Van Ree et al., 1999). The seed capsule of the poppy contains morphine, codeine and other alkaloids. The clinical benefits of opioid as a pain-relief agent led to the development of synthetic opioid medications such as methadone, heroin and fentanyl (Linneman et al., 2000; Walwyn et al., 2010). Thus, the term opiates arise in describing any of the psychoactive opioid compounds with opioid-agonistic function (Van Ree et al., 1999). Opioids exert their effects by binding to opioid receptor subtypes (*i.e.* µ-, δ- and κ- receptors) that are distributed in the central and peripheral nervous systems (Quock et al., 1999).

The opioid-like properties of mitragynine was assessed using the twitch contraction of guinea pig ileum induced by electrical stimulation (Watanabe et al., 1997). The inhibitory effect of mitragynine on gastric acid secretion was well demonstrated in rats when injection of mitragynine into the fourth cerebroventricle inhibited the 2-deoxy-$_D$-glucose-stimulated gastric acid secretion. Interestingly, this inhibitory effect was reversed by naloxone, suggesting the inhibitory effects

possessed by mitragynine were mediated by the opioid system which was similar to morphine (Tsuchiya et al., 2002). Besides, the opioid receptor agonistic effect of mitragynine was also reported in other studies (Matsumoto et al., 1996b; Watanabe et al., 1997; Yamamoto et al., 1999; Boyer et al., 2008; Taufik Hidayat et al., 2010). In addition, mitragynine was also reported to produce antinociceptive activity through supraspinal opioid receptors when its antinociceptive activities following intraperitoneal (i.p.) and intracerebroventrical (i.c.v.) administrations were antagonised by i.c.v. naloxone in both tail-pinch and hot-plate tests (Matsumoto et al., 1996b).

Besides, competitive binding studies showed the involvement of three types of opioid receptors in mitragynine effect, which include μ-, κ- and δ- opioid receptors (Yamamoto et al., 1999; Boyer et al., 2008; Taufik Hidayat et al., 2010). From the three receptor subtypes, mitragynine was shown to exert most of its effects via μ-opioid receptor (Matsumoto et al., 1996b; Thongpradichote et al., 1998; Shamima et al., 2012; Stolt et al., 2014). In addition, receptor binding assays also demonstrated that 7-hydroxymitragynine bound preferentially to μ-opioid receptors, suggesting its full agonistic properties (Takayama et al., 2002; Matsumoto et al., 2004). Taken together, mitragynine and its 7-hydroxymitragynine derivative are reported to act on the central nervous system (CNS) and exhibit high affinities toward μ-opioid receptor (Yamamoto et al., 1999; Matsumoto et al., 2006).