PHARMACOKINETIC AND PHARMACODYNAMIC MODELING OF MITRAGYNINE IN PAIN TOLERANCE

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by

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LIST OF SYMBOLS AND ABBREVATIONS

µg	micrograms
µl	microliter
ACN	Acetonitrile
ALT	Alanine aminotransferase
AMP	Amphetamine
AST	Aspartate aminotransferase
BSV	Between subject variability
BZD	Benzodiazepines
C_{18}	Octadecyl type hydrocarbon with 18 carbon atoms bonded silica stationary phase
CDR	Centre for Drug Research
CI	Confidence Interval
CL/V	Clearance per volume
C_{max}	Peak plasma concentration
COWS	Clinical Opioid Withdrawal Scale
COX	Cyclo Oxygenase
CPT	Cold Pressor Task
CV	Coefficient of variation

CWRES	Conditional weighted residuals
Corr	Corrected
DOA	Drug of Abuse
DU	Duodenal Ulcer
DV	Obtained Observation
EDTA	Ethylene Diamine Tetra Acetate
ESI	Electron Spray Ionization
FOCE	First-Order Conditional Estimation
GOF	Goodness of Fit
GFR	Glomerular filtration rate
HPLC-DAD	High Performance Liquid Chromatography coupled Diode Array detector
ID	IDentification
IWRES	Individual Weighted Residuals
IIV	Inter- individual variability
IPRED	Individual predicted values
K	Ketum
K12	Distribution
K21	Redistribution

Ka	Absorption rate constant
Kp	Absorption rate constant of plasma
KET	Ketamine
kg	kilogram
LCMS/MS	Liquid Chromatography coupled with Mass Spectroscopy
LIT	Linear Ion Trap
MS	Mass Spectroscopy
METH	Methamphetamine
mg	milligram
MG	Mitragynine
min	minute
ml	millilitre
MOR	Morphine
MRM	Multiple Reaction Monitoring
MTD	Methadone
NaOH	Sodium Hydroxide
ng	Nanogram
NLME	Nonlinear mixed effect

nm	nanometre
NONMEM	Non-linear mixed effect modeling
OFV	Objective Function Value
P	Placebo
PKPD	Pharmacokinetics and Pharmacodynamics
pcVPC	prediction corrected Visual Predictive Check
PD	Pharmacodynamic
PE	Prediction Errors
pH	Negative logarithm of H+ concentration
PK	Pharmacokinetic
PRED	Population predicted values
QC	Quality Control
r^2	correlation coefficient
RCT	Randomized Controlled Trial
RDBPC	Randomized Double-Blind Placebo-Control
RPCT	Randomized Placebo Controlled Trial
rpm	revolutions per minute
SE	Standard Errors

SPE	Solid Phase Extraction
T	Time
$T_{1/2}$	Elimination half life
TAD	Time after dose
THC	Tetrahydrocannibinoid
T_{max}	Time to reach peak plasma concentration
UVF	Ultrapure Water Purifier
Vd	Volume of Distribution
VPC	Visual Predictive Check

PEMODELAN FARMAKOKINETIK DAN FARMAKODINAMIK MITRAGININA DALAM TOLERANSI KESAKITAN

ABSTRAK

Mitragyna speciosa, yang dikenali sebagai 'Ketum' atau ' Biak-biak' di Malaysia dan Kratom di Thailand, merupakan sejenis tumbuhan asli yang boleh terdapat di Asia Tenggara. Ia mempunyai sejarah penggunaan sebagai ubat tradisional dalam kalangan penduduk Asia Tenggara dan terkini, Ketum telah mendapat perhatian di negara-negara Barat. Mitraginina (MG) merupakan alkaloid utama yang terdapat dalam daun Ketum (*Mitragyna speciosa),* laporan daripada beberapa kajian telah membuktikan bahawa MG adalah alkaloid yang bertanggungjawab untuk tindakan terapeutik dalam tumbuhan tersebut. Di antara kesan Ketum yang telah dikenal pasti melalui laporan kendiri pengguna Ketum ialah ia telah terbuktikan melegakan kesakitan. Walau bagaimanapun, tidak terdapat kajian klinikal yang dilakukan untuk melaporkan ciri-ciri analgesik Ketum menggunakan satu formulasi yang seragam. Dengan ini, satu kajian penyelidikan eksperimental telah dijalankan secara sukarela di Universiti Sains Malaysia, bertujuan mengkaji hubung kait antara penggunaan Ketum dengan toleransi kesakitan secara rawak, rabun dwi-pihak dan kawalan-plasebo. Minuman Ketum dan plasebo telah disediakan dan diseragamkan sebagai satu formulasi yang akan diberi kepada peserta dalam kajian klinikal. Kaedah analitikal kromatografi cecair berprestasi tinggi bersama pengesan dioda “array” yang telah disahkan (HPLC-DAD) digunakan bagi mengkuantifikasi kadar mitraginina dalam minuman sejumlah 1.6 mg/kg, untuk diminum oleh peserta. Kajian seramai dua puluh enam lelaki warganegara Malaysia, min (SD) umur 24±3 tahun, telah didaftarkan

dalam kajian klinikal ini. Minuman Ketum atau plasebo telah dipadankan dari segi rasa dan penampilan antara dua minuman tersebut. Kemunculan kesakitan dan toleransi telah diukur secara objektif menggunakan 'cold pressor task' (CPT) dalam 'Libert Scale Grading' yang diambil sebagai penanda akhir PD. 'Clinical opioid withdrawal scale' (COWs) telah digunakan untuk menilai sebarang gejala penarikan balik dan tanda-tanda penting. Untuk kajian PKPD, sampel plasma telah diambil dari kesemua 26 perserta kajian. Kajian LCMS/MS yang telah disahgunakan menganalisa kesemua sampel plasma yang diperolehi dari kajian-kajian klinikal. Kedua-dua kaedah HPLS- DAD dan LCMS/MS yang telah disahgunakan mengukur jumlah MG di dalam rebusan ketum dan sampel plasma. Toleransi kesakitan meningkat secara signifikan selepas 1 jam pengambilan ketum dengan purata (SD 11.2 (±6.7) saat segera sebelum 24.9 (±39.4) saat dengan pengambilan (F(253.7 = 4.33, p = 0.02)). Selepas 1 jam pengambilannya, rebusan ketum memperagakan kehilangan kesakitan. Sepanjang kajian klinikal ini, dilaporkan tiada tanda-tanda ketidakselesaan, gejala ganjil atau tanda-tanda penarikan balik ketum. Hubungkait farmakokinetic dan farmakodinamik alkaloid aktif MG menggunakan model ukuran penanda akhir dan toleransi kesakitan. Model 1 kompartmen telah dijelaskan model paling tepat/sesuai untuk menerangkan PK MG. Parameter PK yang diperolehi adalah kadar cepat penyerapan (1.56 ±0.24 h^(-1)), taburan isipadu (99.9 ±10.7L/h) dan klearans (11.9 ±1.27L). Satu hubungkait dos-tindakbalas yang lelurus diperhatikan untuk toleransi kesakitan. Besar kemungkinan, kajian ini menyokong penggunaan rebusan ketum sebagai ubat mengawal kesakitan kronik secara pengambilan kendiri. Bagaimanapun, kajian seterusnya memerlukan saiz sampel yang lebih besar untuk profil efikasi dan keselamatan penggunannya.

PHARMACOKINETIC AND PHARMACODYNAMIC MODELING OF MITRAGYNINE IN PAIN TOLERANCE

ABSTRACT

Mitragyna speciosa, also known as 'Ketum'or 'Biak-biak' in Malaysia and Kratom in Thailand, is a native plant to Southeast Asia. It has a long history of folk medicine use in Southeast Asia and recently has gained popularity in Western countries. Mitragynine (MG) is the principal alkaloid found in the leaves of *Mitragyna speciosa* and has been reported to be responsible for the plant's therapeutic actions. Pain relief is among many self-reported beneficial kratom effects. However, there have not been any controlled clinical studies reported on the analgesic property of Ketum employed a standardized formulation. The objective of this study is to evaluate the PKPD of mitragynine in human subjects. In view of this, work was undertaken in Universiti Sains Malaysia to scientifically assess the pain relief effects of Ketum decoction in a randomized, double-blind, placebo-controlled study. Ketum and placebo decoctions were prepared and standardized as a formulation to be administered to the subjects in the clinical study. A validated HPLC-DAD method was employed for the quantification of mitragynine in the decoction. This was done to ensure that a fixed Ketum decoction dose of 1.6mg/kg (Mitragynine) is given to subjects on the trial. Twenty-six Malaysian males (chronic Ketum users), mean (SD) age 24±3 years, participated in this study. Both Ketum decoction and placebo were matched for tastes and appearances. Pain onset and tolerance were measured objectively using a cold pressor task (CPT) in Libert Scale Grading which was taken as the PD endpoint. Clinical Opioid Withdrawal Scale (COWS) was used to assess any possible withdrawal

symptoms and also vital signs. For PKPD modeling purposes plasma samples were drawn from all 26 subjects involved in this study. A validated LCMS/MS was employed to analyze plasma samples obtained from clinical studies. Both HPLC-DAD and LCMS/MS methods were validated and successfully applied to quantify mitragynine in Ketum decoction and plasma respectively. Pain tolerance increased significantly 1 hour after Ketum ingestion from the mean (SD) 11.2 (6.7) seconds immediately before to 24.9 (39.4) seconds 1 hour after Ketum consumption ($F(2, 53.7) = 4.33$, $p=0.02$). With regards to clinical evaluation, Ketum decoction demonstrated pain relief effect one hour after the ingestion. There were no discomfort, unusual symptoms, or signs of withdrawal symptoms were reported or observed throughout the clinical trials. A population and the individual pharmacokinetic and pharmacodynamic relationship of the active alkaloid mitragynine were modeled using the endpoint measurement or pain tolerance. One compartment model showed the best fit to describe the mitragynine disposition kinetics. The pharmacokinetic parameters established were the absorption rate constant (1.56 ± 0.24 h^{-1}), volume of distribution (99.9 ± 10.7 L/h) and clearance (11.9 ± 1.27 L). A linear dose-response relationship was observed for pain tolerance. This study possibly supports the use of Ketum decoction in self-management intervention of chronic pain. However, the study warrants further investigation employing a larger sample size for its efficacy and safety profiles.

CHAPTER 1

INTRODUCTION

Over the years, herbal plants have been the target of many researchers for their medicinal values. Plants are the natural resources of pharmacologically active compounds (Shukla et al., 2010; Vieira et al., 2014). In this regard, alternative and traditional medicine practices (Traditional Chinese Medicine (TCM), Ayurveda, Homeopathy (or) Siddha medicine) play a vital role in meeting the fundamental health care needs of the population and distinct features of these medicines in many developing countries. (WHO, 2013).

Ethnomedicine is an area which can be explored in depth in Malaysia because it has large biodiversity of natural products; there are tropical plants of about 550 genera and 1,300 species with therapeutic properties. Many Malaysian plants studied produce clinically effective drugs such as *Centella asiatica* (pegaga), *Andrographis paniculata* (hempedu bumi), *Datura mete* (kecubung), *Areca catechu* (pokok pinang),*Ricinus communis* (jarak), and *Strychnos nuxvomica L* (unknown local name) (Jamal, 2006). *Strychnos nux-vomica L* is known for its stimulating effects on central nervous system (CNS) and *Datura mete* for its sedative properties (Jamal, 2006). One of the local plants that earned much attention currently is the *Mitragyna speciosa*. (Korth) which produced psychotropic effect. It is usually recognized in Malaysia as Ketum or Biak-Biak and in Thailand as Kratom (Hassan et al., 2013, Singh et al., 2016). Traditionally, people in rural areas of Malaysia and Thailand use Ketum leaves as a folk remedy for a broad range of conditions/ailments including treating muscle pain, intestinal infection, cough, diarrhea, and fever, anti-depressant and as an appetite-suppressing agent (Hassan et al., 2013). Besides its known healing properties, manual labourers (e.g. farmers, rubber tappers, machine operators, and

drivers) ingesting/taking Ketum to enhance their physical endurance due to their laborious work and to combat fatigue. However, at the same time this indigenous plant of Southeast Asia is widely used by addicts to wean off from opiate withdrawal effects (Singh et al., 2016). The Ketum leaves have been discovered to have approximately 40 alkaloids. The alkaloids content in the leaves depends on the geographical regions and the season of harvest (Shellard 1974). Mitragynine (MG), is the principal alkaloid reported for pharmacological effects. Watanabe et al., (1997) demonstrated the binding of mitragynine to opioid receptors in guinea-pig ileum despite not having morphine-like structure. Interestingly, its minor constituent 7-hydroxy mitragynine inhibited electrically induced contraction through opioid receptors in guinea-pig ileum, and its effect was about 13-fold more potent than morphine (Takayama et al., 2002; Matsumoto et al., 2004). There have been studies reporting the receptor-binding assays, which demonstrated that 7-hydroxy mitragynine had greater affinity to μ-opioid receptor compared to the other opioid receptors (Takayama et al., 2002; Matsumoto et al., 2004). Ketum extracts and mitragynine have been widely reported for their anti-nociceptive activity in animals (Babu et al., 2008). It's binding to opioid receptors could possibly explain the anti-nociceptive activity in animals' studies (Babu et al., 2008; Reanmongkol, Keapraduh & Sawangjaroen, 2007; Sabetghadam, Ramanathan & Mansor, 2010).

Interestingly in Malaysia, in spite of all these preclinical scientific data supporting the analgesic properties of Ketum, heroin addicts' use Ketum leaves as a pain remedy and to wean off from opiate withdrawal effects (Vicknasingam et al., 2010). Further to this, Ketum has gained international prominence in Europe and US, where it was reported to be used for self-management of pain-related various medical conditions and addiction as well

(Singh et al., 2016 Prozialeck, 2016, Saingam et al, 2013). Ketum preparations are sold in the US and several European countries through online merchants or in physical retail stores in different forms (e.g. dried or powdered leaves in bulk or in capsules, tablets, and other forms of extracts). The use of Ketum has been banned in Malaysia due to its abuse by opiate users (Vicknasingam et al., 2010). Oftentimes, Ketum misuse and abuse occur often times especially when it is adulterated with other substances (Kronstrand et al., 2011; Singh et al., 2016). Adulterants include cough syrups, traditional herbs and even synthetic pyrethroid obtained from mosquito coil. This combination could increase the zest, which may have contributed to severe problems (Bothiphon et al., 2009). This has led to regulatory agencies negative perception of Ketum even though traditionally this plant had been used for its therapeutic values for decades.

However, recent self-reporting studies on Ketum users did not reveal any severe addictive effects after long term exposure of this plant decoction (Singh et al., 2016). To date, no death has been reported in this region after regular consumption of Ketum. In the traditional settings, villagers consumed Ketum in the form of decoction. These are used for coping, enhancement and social motives (Singh et al., 2019). In other regions (US & Europe) Ketum products are available in the form of dried powdered leaves, capsules, tablets and other extracts for self-management of pain (Kronstrand et al., 2011; Singh et al., 2016).

The medicinal values of Ketum could not be undermined though it is open to abuse. Currently, the existing self-reporting data on Ketum pain management are inadequate to substantiate their claims on Ketum analgesic properties (Prozialeck, 2016). Based on these findings and in order to evaluate the analgesic properties of Ketum, a randomized

controlled clinical trial involving individuals with previous exposure to Ketum are needed to verify the claims.

The clinical efficacy of drug therapy is determined by its pharmacokinetic (PK) and pharmacodynamic (PD) properties. As of to date, preclinical studies on Ketum and mitragynine are limited; and studies on their PKPD relationship are lacking. With regards to clinical studies, only the Thai's reported mitragynine pharmacokinetic in chronic Ketum users after the subjects had received the Ketum decoction (Trakulsirichai et al., 2015) In this study, Ketum/mitragynine analgesic properties were not investigated. Hence, a rational approach is warranted for accurate prediction of dose-response relationship; as this is essential for the development of Ketum/ MG as an alternative medicinal drug for pain management.

In this study, chronic Ketum users were randomly assigned to a double-blinded placebo-controlled clinical trial to investigate the analgesic effect of Ketum decoction. MG is the principal alkaloid of Ketum, was employed to establish PKPD model. This PKPD model could provide meaningful information on selecting the safe and effective Ketum doses for future pain studies

1.1 Problem Statement

Various studies have reported that pain relief is the main reason for Ketum use in both Southeast Asia and US/Europe regions (Singh et al., 2016; Hassan et al., 2013, Prozialeck, 2016). The latter is more to manage pain related to medical conditions and addiction. However, the existing interview/surveys and self-reporting studies of Ketum users are only anecdotal evidences supporting Ketum's pain relief effect. As to the best of our knowledge, there is no controlled clinical study to support such claims. Ketum leaves contain multiple alkaloids, however mitragynine is considered as the primary active substance responsible for ketum pharmacological effects. To date, preclinical studies on mitragynine are limited; and only one study enrolling human subjects reported mitragynine pharmacokinetic in chronic ketum users (Reanmongkol, Keapraduh & Sawangjaroen, 2007; Sabetghadam, Ramanathan & Mansor, 2010; Trakulsirihai et al, 2015).

In view of this, the pharmacokinetic/pharmacodynamic profiles of mitragynine plasma levels were obtained during a randomized, double-blind, placebo-controlled laboratory study enrolling a sample of ketum users. For the first time we report a clear relationship between plasma mitragynine levels and pain tolerance, which was quantified with a population PKPD model.

1.2.1 Main Objective

1. To evaluate the PKPD modeling of *Mitragyna speciosa* in pain tolerance in human subjects.

1.2.2 General Objectives

1. To determine the mitragynine content in Ketum decoction using a validated HPLC/DAD method.
2. To determine mitragynine concentration in plasma samples obtained from the clinical study using a validated LCMS/MS method.
3. To determine the extent of pain tolerance (the maximum level of pain that the participant is able to tolerate) as the pharmacodynamic end-point in chronic Ketum users after ingestion of Ketum decoction using the cold pressor task technique.
4. To model the Pharmacokinetic / Pharmacodynamic relationship of mitragynine in chronic Ketum users after having received the Ketum decoction in a Double-Blind Placebo Control study using non-linear fixed effect modeling (NONMEM)

CHAPTER 2

LITERATURE REVIEW

2.1 The role of plant as medicine to human health

Plants have been a major source of food and medicine for humanity. While we all consume plants and their nutritional products, most of the world's population also rely on botanical remedies to meet their health needs, either as their own "traditional medicine" or as "complementary and alternative medicine." (World Health Organization. WHO Traditional Medicine Strategy, 2013, Jonas et al., 2012). Today, in the interest and use of plant-based therapies and botanical health products, we are witnessing a global rejuvenation. The increased interest of common people in herbal medicine and products has stimulated a greater scientific awareness of the pharmacologically active constituents of medicinal plants in exploring and understanding them.

In drug discovery process, natural products remain as a viable and important source of relevant compounds. Many compounds obtained from plant sources have been known to have bio / pharmacological activities and historically plants have produced many important drugs for human use from the pharmaceutical point of view, starting from morphine discovered in the early nineteenth century to paclitaxel and artemisinin in recent years. This development of plants as natural products for human health have their specific roles in the chemical characterization, *in vitro* and *in vivo* activities, clinical effects, mechanism of action, structure-activity relationship and PK/PD properties.

There have been a number of high quality publications around the world. A wide range of disease targets such as diabetes, inflammation, cancer, neurological disease, cardiovascular

disease, liver damage, malarial, bacterial and fungal infections have been published which had significant findings.

The following reviews have been published on natural products in the recent years. They cover various biomedical areas including antimalarial (Pan et al., 2018), anti-arthritic (Dudics et al., 2018), hair growth stimulation (Choi, 2018) Two other reviews have focused on the medicinal plants such as Copaifera (da Trindade et al., 2018) and Citrus species (Dosoky and Setzer, 2018). A review by Thomford et al. discussed the potential for the next generation of plant-based drug discovery by applying innovative technologies such as automation technology, analytical and computational techniques (Thomford et al., 2018).

Preclinical and clinical investigations concerning their mechanisms of action, safety and efficacy are warranted in order to have a better understanding on their medicinal properties and to establish stronger evidence of potential for further development of plants as medicinal products.

In developing nations, over 80% of the people, particularly in rural populations, rely on medicinal plants as part of their primary health care where biomedicines are difficult to obtain (Farnsworth, 1993). The use of plants with medicinal values according to original and biomedical standards can be considered *in lieu* of comparably effective pharmaceuticals. Licorice (*Glycyrrhiza glabra*), myrrh (Commiphora species) and poppy capsule latex (*Papaver somniferum*) are plants first reported for such a practice. The clinically used drugs from the opium plant, *P. somniferum* are papaverine, codeine, morphine and noscapine (narcotine) (Newman et al., 2000). The narcotic analgesic property of the plant affecting the function of central nervous system (CNS) in easing intense pain, made the opiates an essential drug for clinical use as potent analgesics.

However the main adverse effect on consuming such drugs is addiction (Vetulani, 2001). At present, drugs that are proven to be as good as morphine for chronic pain management are very limited. Another psycho active compound with good analgesic effect and potential to treat neurological illnesses are cannabinoids derived from *Cannabis sativa* (Watts, 2004; Fernadez-Ruiz et al., 2007).

Being narcotic, addictive and other severe adverse effects has limited its utilization and therefore its usage is made illegal in many countries. The cannabis plant (known as marijuana, ganja and has many other street names) is widely abused as a recreational drug (Watts, 2004). Other alternatives to morphine drugs such as nalbuphine, pentazocine and butharphanol are clinically available but their actual analgesic properties remain disputed (Petrovic, 2002).

There are many more drugs derived from plants which have been successfully established as pharmaceuticals are not covered in this section. The research in scientific area of ethnomedicicne growing tremendously particularly in country like Malaysia where natural resources are in abundance. One of the plants which require immediate attention is *M. speciosa*. It is widely abused for its narcotic-like effects when smoked, chewed or taken in its decocted form known as "Air Ketum". Despite being widely abused there are also numerous reports on its medicinal values such as anaesthetic, anti- nociceptive, analgesic and psycho stimulant effects (Jansen &Prast 1988; Macko et. al., 1972; Matsumoto et al., 1996 ; Perry, 1980; Tsuchiya et al., 2002). *M. Speciosa* leaves are known for its rich alkaloids content and MG is one of the main alkaloid responsible for the diversified pharmacological properties of the plant. Contrarily, the presence of other chemical

constituents other than alkaloids aren't well documented to explain the anti-infective properties of *M. speciosa* leaves.

2.2- *Mitragyna speciosa*

2.2.1 Description of the plant

The *Mitragyna speciosa* plant known locally as Ketum or Biak-Biak in Malaysia (Hassan et al., 2013, Singh et al., 2016) and in Thailand as Kratom, is a member of the Rubiaceae family. This plant is native to Southeast Asia, countries such as Malaysia, Thailand and Myanmar etc. This plant also called as Kakuam, Thom and Ithang different south-east Asian regions. The name "Korth" belonging to the genus of this plant was named after William Korthal, who is a botanist that found the flower's stigma similar to a bishop's mitre (Shellard, 1974). The taxonomy of the plant is given in Table 2.1 (Eisenman et al, 2014)

Table 2.1- Taxonomy of *Mitragyna speciosa* Korth

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Gentianales
Family	Rubiaceae
Subfamily	Cinchonoideae
Tribe	Naucleeae
Genus	*Mitragyna*
Species	*Speciosa*

2.2.2 Traditional use of *Mitragyna speciosa*

Local people in the community have traditionally used ketum leaves for its curative medicinal properties, and coca-like effects to enhance work tolerance against laborious work under the blazing sun (Suwanlert, 1975; Singh et al., 2016). Ketum was also used as an opium substitute in Malaya and in Thailand to wean off morphine addicts (Jansen & Prast, 1988). With accumulating interest and rising potential from perceived therapeutic benefits of ketum, it can be inferred that this indigenous medicinal plant of Southeast Asia has huge potential to be used as an analgesic. Besides its healing properties, manual laborers (e.g. farmers, rubber tappers, machine operators, and drivers) also reported ingesting/taking ketum to enhance their physical endurance to laborious work and to combat fatigue (Vicknasingam et al., 2010)

2.2.3 Pharmacological properties of *Mitragyna speciosa*

The clinical efficacy of a drug therapy is determined by its PK and PD profiles. Even though the pharmacological effects of ketum are established in human and experimental animals, the PK's and toxicity studies with regards to pure MG and ketum extracts with the different alkaloids still remains poorly defined.

Extensive research has been conducted over the last few decades to understand the pharmacological effects of *M.speciosa* and its active alkaloid mitragynine in animal experiments (Hassan et al., 2013). Off late Trakulsrichai et al (2015) reported the first PK properties of mitragynine in regular ketum users.

2.2.4 Phytochemistry and pharmacological studies of *Mitragyna speciosa*

There are about 25 different alkaloids in *M.speciosa* – mitragynine and 7-hydromitragynine as both the major and minor constituent were reported to have similar pharmacological characteristics as opioid agonist (Hassan et al., 2013). *M.speciosa* preparation and mitragynine have proven to produce narcotic effects which are dependent on the dose employed where stimulant effects were noticed at lower doses and higher dose produced sedative effects. Matsumoto et al. (1996a) found mitragynine have anti-nociceptive action through the supraspinal opioid receptors as its action is dominantly mediated by μ and δ receptor subtypes *in vivo* and *in vitro* studies. Its minor constituent 7-hydroxymitragynine was reported to exhibit a higher potency than mitragynine in the guinea-pig ileum test and have a high affinity to μ opioid receptors in bindings assays (Takayama et al., 2002). However 7-hydroxymitragynine was found to be structurally different from other opioid agonists and has a more potent anti-nociceptive activity than morphine, especially after oral administration (Matsumoto et al., 2004). Takayama (2004) reported 7-hydroxymitragynine have high affinity to opioid receptors, with 13-fold and 46-fold higher potency than morphine and mitragynine respectively.

Many analytical approaches have been reported for the separation and identification of ketum, including GC and GC-MS (Kapp et al., 2011) and CE and CE-MS (Posch 2012, Fu & McCord, 2013, Fu, 2014). A UV spectrophotometer approach and HPLC-UV method were employed to ascertain the physicochemical properties of mitragynine (Parthasarathy et al., 2015). This study has pointed out that mitragynine is a weak base, with intermediate lipophilicity, poor solubility in water and basic media, high solubility in acidic media, and yet acid degradable. Besides, a high-performance liquid chromatographic (HPLC) method

was used for determination of mitragynine in rat serum to study its PKs (Janchawee et al., 2007, Parthasarathy et al., 2010). The PKs evaluation of mitragynine, 7-hydroxymitragynine, and mitraphylline were also performed by LC-MS (Le et al., Parthasarathy et al., 2013, de Moraes et al., 2009). However, the information about the metabolism of mitragynine is limited where the identification of phase I and II metabolites of mitragynine in rat and human urine using liquid chromatography-mass spectrometry (LC-MS) coupled with a linear ion trap (LIT) analyzer which gives a detailed information on the structure in the MSn mode and these finding is confirmed with the high resolution mass spectrometry with an Orbitrap (OT) analyzer, where the empirical formula of the corresponding fragments will be provided (Phillip et al.,2009).

One of the limitation for the quantifation of mitragynine is the stability issue of these compound. The stability of mitragynine at concentrations 5.0 and 50.0µg/ml in methanol were evaluated at storage temperature (4˚C) over a period of 4 weeks using HPLC technique. Result of the stability study showed that mitragynine was stable in methanol over a period of one month (Parthasarathy et al., 2007). The stability in the urine sample is measured by analyzing two concentrations (low QC and high QC) of analytes using LC-MS technique. The urine standards were stored at 4 °C and were found to be stable for 2 weeks (Fu et al., 2015). These studies did not provide stability of mitragynine at different time periods and at different temperatures. In addition, accelerated stability studies have not been reported for the mitragynine pure compound.

2.3 Physicochemical properties/characterization of mitragynine

There should be an absolute understanding on the relevant therapeutic and physicochemical properties of the drug which is essential to obtain the desired formulation and drug delivery

system. In the physicochemical section, the two most relevant physicochemical properties to drug delivery, solubility and stability, are discussed.

2.3.1 Solubility and Stability studies

When the aqueous solubility of a drug is less than 1mg/ml there produce significant changes in the absorption parameter. There is a high significance in the stability studies of a drug because upon detoriation, it will lead to reduction in the therapeutic effect and toxic degradation of the product (Ertel & Carstensen, 1990). Yonemochi et al. (1999). In the work of Ramanathan et.al, (2015), mitragynine was indicated as a weak base, with moderate lipophilic property. It was also found that this compound was poorly soluble in water and alkaline medium, highly soluble in acidic medium, but acid degradable (Ramanathan et al., 2015). The stability study which was performed *in vitro* using simulated gastrointestinal fluid model found that mitragynine was unstable in SGF (simulated gastric fluid) and stable in SIF (simulated intestinal fluid) but shows poor solubility in SIF (Ramanathan et al., 2015, Manda et al., 2014).

Kong and colleagues (2015) discovered that *M. speciosa* alkaloid extract (MSAE) and mitragynine are highly soluble in aqueous phase at pH 4.0 and moderately soluble at pH 7.4. However, the passive diffusion across the phospholipid bilayer membrane was higher at pH 7.4, and the contrary at pH 4.0. In addition, plasma protein binding and metabolic stability of mitragynine were found high in rat liver microsomes (Kong et al., 2017). Besides mitragynine having the prospective use as an opioid substitute, the equilibrium between potential for abuse and drug efficacy should be considered.

2.3.2 Permeability studies

Drug solubility and permeability are the main features of drug bioavailability. The oral bioavailability of the drug can be evaluated by determining the intestinal permeability of the drug, which is one of the important requirement. Recently, Jagabalan et al. (2018) reported that mitragynine at a dose of 40 µg/ml has high intestinal permeability in rats using *in situ* absorption model. They also found that mitragynine was not affected by P-glycoprotein efflux effect and cytochrome P3A4 which are associated with low doses (Jagabalan et al., 2018). From the above studies, mitragynine showed high solubility in acidic environment, but shows better permeability across intestinal membrane at basic condition.

2.3.3 Nonclinical Vehicle Use in studies

The use of appropriate vehicles to solubilize drug or extracts prior to oral or parental administration is vital in preclinical animal studies. Any drug to be absorbed must be present in the form of solution at the site of absorption; hence various vehicles were used to facilitate the solubility of MG. The vehicles commonly used to facilitate the solubility of MG were 1% cremophor in saline, 1% acetic acid (pH 4.7) or propylene glycol, 4% acacia gum, Tween 20, Tween 80 (Janchawee et al., 2007, de Moraes et al., 2009, Trakulsirichai et al., 2015). In most instances upon solubilizing, the drug either in the form of solution or suspension depending upon the dosage employed in preclinical studies. However owing to its basicity suboptimal physicochemical properties, this drug predominately exist in ionic form in gastric juice and encounter solubility issue in intestinal fluid. Thus, there are high possibility of MG being aqueous, basic in nature and moderately lipophilic. Apart from solubility, intestinal permeability of a drug also attributes to its

bioavailability. In case of MG, it showed high intestinal permeability in animal model. On average, the sub-optimal physiochemical properties of MG and differences in vehicles utilized may contribute to the higher variation and conflicting qualities in PK-parameters.

2.4 Pharmacokinetics of mitragynine

PKs is proposed to study the absorption, the distribution, the biotransformation and the elimination of drugs in man and animals. A kinetic profile may be well summarized by C_{max} (maximum concentration), t_{max} (time take to reach C_{max}), $t^{1/2}$ (half-life) and AUC (Area Under Curve) by having more than one profile, 8 parameters at least, the mean and standard deviation of these factors, shall very well outline the kinetics of the drug in the entire populace (Yuen et al, 2010). A detailed description of the data can be obtained interpolating and extrapolating the drug concentrations with some mathematical functions. These functions may be used to reduce all the data in a small set of parameters, or to verify if the hypotheses incorporated in the functions are confirmed by the observations. In the first case, we can say that the task is to get a simulation of the data and in the second to get a model. The multi exponential function helps in the interpolation and in reducing the PK data whereas the compartment models are for the reference whose results are similar to multi exponential functions. The functions used are the multi exponential functions and the reference model whose solutions are just the multi exponential functions. By the use of models, new essential PK parameters can be described which can then be used to correlate the physiological process and the PK profile of the drug which drives the absorption, distribution and elimination of the drug. For instance, compartmental model defines volume of distribution which is dependent on the volume of distribution of the drug in the tissues or the rate of clearance of a drug which is reliant upon the elimination process of

the drug (Kanji et al., 2015). Models also provide an easy way to get an estimate of drug absorption after an extra vascular drug administration (bioavailability). Model building is a complex multistep process where with experiment by experiment and simulation by simulation, new hypothesis were proven and disproven through a continuous interaction between the experimenter and the computer (Kanji et al., 2015).

Till date, the PKs of mitragynine have been studied mostly in rat models after oral and intravenous administration and only one study was conducted by S.Trakulsrichai et al. (2015) on humans.

Mitragynine content was first determined by Janchawee et al. (2007) in the plasma of rats by using a high performance liquid chromatography method with ultraviolet detection (HPLC–UV). In this study, the PK parameters of mitragynine (40 mg/kg) following the oral administration (p.o) in rats were reported from a non-compartmental analysis. The peak plasma concentration (C_{max}) of this dose was 0.63µg/mL with the time taken to reach maximum concentration (T_{max}) is 1.83 hr and an absorption rate constant (ka) of 1.43 ± 0.90 h^{-1}.). The elimination process was considered to be slow because the elimination rate constant (λz) was 0.07 h^{-1} and the clearance (CL) was 1.60 L/h/kg (Janchawee et al., 2007). Following that, HPLC and tandem mass spectrometry (LC–MS/MS) method were developed by de Moraes et al. (2009) to detect mitragynine in rat plasma. Oral administration of mitragynine at 20 mg/kg resulted to a C_{max} of 0.423ug/mL after a T_{max} of 1.26 hr. The total CL and elimination half-life ($t_{1/2}$) was 6.35 L/h/kg and 3.85 h, respectively. Even after 24 hr, mitragynine could still be detected in the plasma samples (de Moraes et al., 2008). Based on the comparison between these studies, mitragynine at high dose contributes to a low clearance rate compared to orally administered low dose.

This indicates that mitragynine at high dose led to delayed clearance which may increase the toxicity profile of mitragynine. Thus, safety dose studies are essential to determine the safe dose of this compound.

In the study of Parthasarathy et al. (2010), the PK parameters were acquired through solid-phase extraction and rapid HPLC–UV analysis. After intravenous administration (iv) of mitragynine at 1.5 mg/kg in rats, the C_{max} was 2.3 ± 1.2 µg/mL after T_{max} = 1.2 ± 1.1 hr. The elimination half-life ($t_{1/2}$) was 2.9 ± 2.1 hr. The CL was 0.29 ± 0.27 L/h/kg. Whereas after oral administration of 50 mg/kg mitragynine in rats, the T_{max} was at 4.5 ± 3.6 hr with C_{max} of 0.7 ± 0.21 µg/ml. The apparent total CL was 7.0 ± 3.0 L/h/kg and the bioavailability was 3.03 ± 1.47% (Parthasarathy et al., 2010). Based on this study, the C_{max} of mitragynine was considered to be low irrespective to the dose administered via oral or iv routes. These findings suggests that there is a possibility of mitragynine being converted to some other metabolites which may contribute to its activity. This warrants further investigation.

Apart from detailed studies on mitragynine's PK profile in animal model, recently S.Trakusirichai and colleagues (2015) reported human PK study with two loading doses of Ketum tea. This was the first study on the PK parameters from 9 existing Ketum users, where time (t_{max}) to reach C_{max} is 0.83 ± 0.35 hour, with terminal half-life at (23.24 ± 16.07 hours), and the apparent volume of distribution (38.04 ± 24.32 L/kg). The unchanged form found in the urine was 0.14% with the clearance rate of 98.1 ± 51.34 L/h/kg. The PKs were observed from oral two-compartment model (Trakulsirichai et al., 2015). The advantages of oral two compartment model are, it gives a visual representation of various rate processes involved in drug disposition. It is also convenient to derive equations describing drug concentration changes in each compartment. The amount of the drug can be estimated

in any compartment of the system after it is introduced into a given compartment whereas the disadvantage is that the type of compartment behavior i.e. the compartment model may change with different routes of administration.

However, this study did not measure the pain effect as pharmacodynamics endpoint to demonstrate a concentration-effect relationship. The overall studies have been summarized in Table 2.2.

Since there are limited animal studies and only one human study on PK of mitragynine, there is a challenge in the selection of appropriate dose for further investigations. From all these PK studies, the range of the dose employed varies significantly. These variations are observed in both preclinical and in human studies as well. Apart from the dosing, different routes of administration and extraction method also contributes to do the variability in PK data.

Table 2.2: Pharmacokinetics of mitragynine

Species	**MG dose (mg/kg)**	**Cmax µg/ml**	**Tmax (hours)**	**t½ (hours)$^{-1}$**	**Clearance L/h/kg**	**Volume of Distribution-Vd/F (L/kg)**	**Authors**
Rats	40 (p.o.)-(Vehicle: 100% propylene glycol)	0.63 ± 0.18	1.83 ± 1.25	9.43 ± 1.74	1.60 ± 0.58	89.50 ± 30.30	Janchawee et al., 2007
	20 (p.o.) (Vehicle: 1% Acetic acid)	0.42 ± 61.79	1.26 ± 0.20	3.85 ± 0.51	6.35 ± 0.43	37.90 ± 5.41	De Moraes et al., 2009
	1.5 (iv)(Vehicle: 20% Tween 20)	2.3 ± 1.2	1.20 ± 1.1	2.9 ± 2.1	0.29 ± 0.27 (absolute)	0.79 ± 0.42	Parthasarathy et al., 2010
	50 (p.o.)(Vehicle: 20% Tween 20)	0.7 ± 0.21	4.5 ± 3.6	6.6 ± 1.3	7.0 ± 3.0	64 ± 23	Parthasarathy et al., 2010

Humans	**<u>Loading Dose (p.o)</u>** (Vehicle: Distilled Water)		0.83 ± 0.35	23.24 ± 16.07	98.1 ± 51.34	38.04±24.32	Trakusirichai et al., 2015
	High:23	0.105					
	Low: 6.25	0.0185					

2.5 Pharmacodynamics of mitragynine (Anti-nociceptive/analgesic)

PDs explains about the action of a drug inside the body when administered which comprises of binding of the drug to a receptor (including sensitivity of the receptor), post-receptor effects, and interactions with chemical mediators in the body. PDs with PKs helps in explaining the relationship between the dose and response, i.e., the drug's effects (Meibohm & Derendorf, 2002). A drug's pharmacological action depends on the extent of its binding to its receptor. The action of a drug is determined by the amount or the concentration of the drug at the site of the receptor. The PDs profile of a drug can be affected by the physiologic changes due to disorders, aging, or other drugs. Certain disorders which alter the PD reactions involve thyrotoxicosis, Parkinson disease, malnutrition, myasthenia gravis, genetic mutations and some forms of diabetes mellitus (Danhof, 2015). These conditions can alter receptor binding, bring down the receptor sensitivity or change the level of binding proteins. Aging affects PD responses by altering the binding of drug to receptor or post-receptor response sensitivity. PD drug-drug interactions either results to alter post-receptor response or competition for receptor binding sites (Richens 1995).

Pain is an experience which is ubiquitous yet at the same time quintessentially subjective. It is influenced by an incredible array of contextual factors including those in the spiritual, social, cultural, cognitive, emotional and bio-medical domains. It is private and is often suppressed, concealed or exaggerated according to the setting. This means that drawing inferences based on the behavior of the person with pain is problematic. This is nothing new to pain researchers and is perhaps part of the reason so many people feel drawn to study it, either to explain the "puzzle" or rise to the "challenge" of pain (Rafaeli, 2017).

Even though Opioids are considered to have consequential adverse effects which hamper their use, they are still opted as the first choice for control of pain and sedation in the Intensive Care Unit (ICU). Long-term opioid use leads to tolerance (i.e., less susceptibility to the effects of the opioid, which can result in a need for higher and more frequent doses to achieve the same analgesic effect), physical dependence, and opioid-withdrawal symptoms during weaning and contributes to the development of chronic pain later and opioid-induced hyperalgesia (a paradoxical hypersensitivity to pain) (Naji et al., 2016). Opioid tolerance can be seen during all types of critical illnesses; the magnitude, however seems to be exaggerated in patients who have had major trauma (e.g., burn injury), in patients requiring prolonged mechanical ventilation, and in pediatric patients. The development of tolerance is because of the larger doses used to control pain in these critically ill patients. However, the inflammatory response to opioids themselves, seen in patients in the medical ICU and those in the surgical ICU, plays an important role in tolerance (Ballantyne, 2017).

With regards to mitragynine, high affinity was found towards κ-opioid receptors followed by 0μ- and δ-opioid receptors (Taufik et al., 2010). It acts as a receptor agonist at μ-opioid receptors and possibly as an antagonist at κ-opioid receptors (Yamamoto et al., 1999, Shamima et al., 2012, Yusoff et al., 2017). At cellular level, mitragynine blocks neuronal Ca^{2+} channels (Matsumoto et al., 2015). It was also found to inhibit forskolin-stimulated cyclic adenosine monophosphate (cAMP) formation *in vitro* in an opiate receptor-dependent way (Tohda et al., 1997, Jamil et al., 2013). Expression of cAMP and cAMP response element-binding protein (CREB) were reported due to repeated exposure of mitragynine (Fakurazi et al., 2013). Twitch contractions of the guinea pig ileum occurred