# Comparison of The Effect of Subacute Treatment of *Mitragyna speciosa* KORTH Standardized Methanol Extract and Morphine on the Development of Antinociceptive Tolerance to Thermal Noxious Stimuli in Swiss Albino Mice

by

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Dissertation Submitted In Partial Fulfillment

Of The Requirements for the

Degree Of Master Of Medicine

(Anaesthesiology)

UNIVERSITI SAINS MALAYSIA

NOVEMBER 2010

# ACKNOWLEDGEMENTS

I wish to express my gratitude, first and foremost, to the Most Benevolent Allah SWT for giving me the blessing and strength in every step of completing this research. My gratitude and appreciation is extended to the following individuals for their continuing support, guidance and technical advices in ensuring the success and completion of this project.

Prof. Sharif Mahsufi Mansor, Center For Drug Research, USM, for his guidance and timely supply of *M. speciosa* Korth methanol extract. Dr. Nizar Abd Jalil, my main supervisor, for respecting my wishes and suggestions on conducting this research. Prof. Dr. Jafri Malin Abdullah, Head of Department of Neurosciences , USM for words of wisdom and insights;

All the lecturers at the Department of Anesthesiology, USM for their encouragement. My anesthetic colleagues at the Department of Anesthesiology, USM, for their support and understanding. Dr Fadzli, Lecturer of PPSG, USM, for his help with the statistics. LARU staff for their help and support.

Lastly, on a personal note, to my parents, mama and babo, and my family, to whom I dedicate this work. Thank you for your never-ending encouragement and pray.

This research had received funding in the form of Research Incentive Grant (304/JPNJ/600004) awarded by Universiti Sains Malaysia in 2010.

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# ABSTRAK

**Latar belakang**

Mitragynine adalah kandungan utama di antara 25 alkaloid yang terdapat di dalam *Mitragyna speciosa* Korth standardized methanol extract. Mitragynine adalah agonist untuk reseptor mu dan delta opioid, seperti morphine, yang memberi kesan tahan sakit terhadap stimulus kesakitan mekanik dan thermal. Kajian ini adalah untuk mengkaji kesan suntikan subacute *M. speciosa* Korth ke atas tikus Swiss albino dan toleransi terhadap kesan tahan sakit. Kajian ini juga membuat perbandingan di antara *M. speciosa* Korth dan morphine.

**Metodologi**

Tikus Swiss albino di bahagikan kepada 3 kumpulan mengikut suntikan yang diterima; *M. speciosa* Korth, morphine dan placebo. Kemudian, tikus tersebut di letakkan di atas hot plate 30 minit selepas suntikan sehingga menunjukkan tanda kesakitan ( melompat, menjilat kaki), selama 6 hari. Hari ketujuh, tikus yang menerima suntikan *M. speciosa* diberi suntikan cabaran *M. speciosa* manakala tikus yang menerima suntikan morphine dan placebo diberi suntikan cabaran morphine. Tetikus tersebut kemudian diletakkan di atas hot plate setiap 30 minit selama 120 minit. Masa tindak balas terhadap stimulus (baseline latency) dikira sebagai peratusan tindakbalas maksimum (%MPE).

**Keputusan**

Tikus yang menerima suntikan M. speciosa Korth menunjukkan peningkatan tahan sakit selepas 6 hari berbanding placebo ($p<0.01$). Tikus yang menerima suntikan morphine pula menunjukkan sedikit pengurangan kesan tahan sakit pada hari ke-6. Baseline

latency untuk kumpulan M. speciosa Korth dan morphine meningkat selepas rawat berbanding sebelum rawatan ($p<0.01$) tetapi tiada perbezaan ketara dia antara kedua kumpulan ($p>0.05$).

**Kesimpulan**

Tikus yang menerima suntikan M. speciosa Korth secara subacute tidak menunjukkan tanda toleransi terhadap stimulus hot plate tetapi menunjukkan peningkatan kesan tahan sakit. Ini berbeza dengan tikus yang menerima suntikan morphine yang menunjukkan sedikit toleransi pada hari ke-6. Walaubagaimanapun, perbezaan ini tidak signifikan secara statistik.

## ABSTRACT

**Background**

Mitragynine is the main constituent of 25 alkaloids contained in *Mitragyna speciosa* Korth standardized methanol extract. It acts on mu and delta opioid receptor as an agonist, similar to morphine and produce analgesic effect in response to both thermal and mechanical pain stimulus. This research was to study the effect of subacute administration *M. speciosa* Korth on development of tolerance, and comparing it to morphine.

**Methodology**

Swiss albino mice were divided into 3 groups, each were subjected to daily doses of either *M. speciosa* Korth, morphine or placebo, given subcutaneously for 6 days. They were subjected to daily hot plate thermal noxious stimuli 30 minutes after administration, until the sign of pain exhibited (hind-paw licking, jumping). On day 7, the *M. speciosa* Korth administered mice were given a single challenge dose of *M. speciosa* Korth while the morphine and placebo treated mice were given morphine and subjected to hot plate test for 120 minutes, at 30 minutes interval. The changes in baseline latencies were also quantified. The antinociceptive response was quantified as percentage of maximal possible effect, %MPE.

**Results**

*M. speciosa* Korth standardized methanol extract administered mice showed increase antinociceptive response by day 6 when compared to placebo ($p<0.01$), while morphine treated mice showed minimal tolerance by day 6. The baseline latencies were

significantly increase for both *M. speciosa* Korth and morphine group post treatment when compared to pre treatment ($p<0.01$), however, the difference between groups were not significant ($p>0.05$).

**Conclusion**

There was no development of tolerance, but an increase in analgesic effect in mice administered with *M. speciosa* Korth methanol extract in subacute duration. In comparison, morphine treated mice showed minimal tolerance in antinociceptive response by day 6, although the different between groups was not statistically significant.

# CHAPTER 1

# INTRODUCTION

## 1.1 Treatment with Opioid Agonist and Development of Tolerance

In clinical practice, Morphine has long been the only effective analgesic available for acute and chronic pain treatment. In acute pain service, the emergence of other class of drugs such as non-steroidal anti-inflammatory drugs, acetaminophen or local anesthetics give treating physicians better options to tailor to patients' need and condition. The concept of multimodal analgesia , that is of combining pain medication of different pharmacokinetic and pharmacodynamic properties, is gaining traction as the standard protocol for acute and chronic pain management. Tolerance will inevitably occurs with repeated administration of opioid drugs, even after few injections in animal study. The importance of continuation of pain treatment even in subacute phase and its potential to reduce the development of the tolerance in antinociceptive action in the chronic pain have yet to be studied. This is crucial as the delay in tolerance development will be helpful in chronic pain management, where long term treatment with increasing doses of morphine as the pathological condition progressed is inevitable. The effect of long term morphine treatment and its subsequent development of tolerance had long been proved in animal study and human research. Besides tolerance, long-term treatment with morphine has many undesirable side effects such nausea and vomiting, constipation, respiratory depression and physical dependence. The study on subacute administration and the development of tolerance have been few and the results were varied, mainly due to difference in definition of subacute phase. Several theories on the mechanism of opioid tolerance development have been proposed based on cellular and

molecular level researches. Of these theories, the superactivation of cyclic Adenosine Monophosphate (cAMP) - dependant protein kinase A signaling cascade has long been recognized as a typical molecular adaptation to chronic opioid administration (Nestler E.J.).

This study focus is to investigate the relationship between increase doses of drugs or active compound of Mitragyna speciosa Korth standardized methanol extract and the development of tolarance to anti-nociception and comparing this with the morphine in the subacute phase of the treatment. It is to this author knowledge that no such comparison study has ever been published previously and hoping that it would serve as a catalyst for the future study using pure compound when it is made available.

### 1.2 *Mitragyna speciosa* Korth

*Mitragyna speciosa* Korth, better know as ketum is an indigenous plant thrive on many densely forested lands in Malaysia (Hashim, 1987). The plant is of *Rubiaeceae* family (Idid et all, 1998). It contains as many as 25 indole alkaloids, of which mitragynine the main constituent (66%) (D. Ponglux, 1994; E.J. Shellard, 1974). While its chemical content had only been recently discovered, *M. speciosa* Korth has long been used for its opioid like effect (Burkill, 1935) and also as treatment for opium addiction (Suwanlert, 1975). The interest in *M. speciosa* Korth increased when Watanabe and Matsumoto et all demonstrated the agonistic effect of mitragynine on opioid receptors (Watanabe et all, 1997, Matsumoto et all, 2005). It is however, structurally different from morphine. These findings propel further research to look into the action of *M. speciosa* Korth extract and its alkaloids on different opioid receptors and their behavioral changes. The main bulk of these researches focused on its antinociceptive effect in acute doses. Previous study by S.Z. Idid et al, 1998, showed an equipotent dosage of *M. speciosa* Korth extract 200 mg/kg to morphine 5 mg/kg given

orally. Other comparison studies on mitragynine and morphine dosage given intra-cerebral ventricle in acute doses showed morphine has 3 times potency (Matsumoto K. et al, 1998). However, to this researcher knowledge, there are no data published on equipotent dose of *M. speciosa* Korthmethanol extract to morphine administered via subcutaneous. Pilot study has been conducted to investigate the equipotent dose of *Mitragyna soeciosa* Korth standardized methanol extract with that of morphine. This study showed that *Mitragyna speciosa* Korth standardized methanol extract at 90mg per kilogram body weight is equivalent to morphine hydrocloride 10mg per kilogram body weight (unpublished data).

**1.3. Thermal noxious stimuli**

Thermal noxious stimulus is the application of heat on the body surface of the animal to evoke pain-related response. This is the most common stimulus used for pain research in any species. In human, the heat evoked flexion reflex has proven to be a good predictor of the analgesic potential of particular pharmacologic compound. The example of the thermal noxious stimuli is hot plate test. A cut off latency time is employed to prevent tissue damage (Kruger et al., 2001)

# CHAPTER 2

# LITERATURE REVIEW

## 2.1. A History Of Morphine as Exogenous Opioid Analgesia

Morphine is the most abundant alkaloid contained in opium plant , Papaver somniferum. It was first discovered in 1804 and distributed in 1817 by Serturner and made commercially available by Merck in 1827. Its usage became widespread after the introduction of hypodermic needle in 1857. It is a potent opiate analgesic and considered to be the prototypical opioids. The structural formula of morphine was determined in 1925 and since then various attempts had been made to produce synthetic morphine. However, the vast majority of morphine is still derived from the opium plants either by traditional method or incorporated processes using various parts of the opium plant. Morphine is a controlled substance under narcotic law in Malaysia. It is also the most commonly abused narcotics until heroin was synthesized. In clinical practice, morphine is one of the gold standard drug used as analgesic for moderate to severe pain, either in management of acute or chronic pain treatment. However, chronic use of morphine causes many undesirable side effects which includes reduced analgesic efficacy or tolerance associated with inevitable increased in dosage, dependency or addiction and withdrawal behavior and symptoms.

## 2.2. Introduction to *Mitragyna speciosa* Korth (*M. speciosa*)

*Mitragyna speciosa (M. speciosa)* korth, better known as ketum is an indigenous plant thrives on many densely forested lands in Malaysia (Hashim, 1987). The plant is of Rubiaeceae family ( Idid et al, 1998) and contains as many as 25 indole alkaloids , of which mitragynine (MG) is the major constituent ( Shellard,1974 ; Ponglux, 1994). *M. speciosa* korth leaves are known to produce opioid-like effect (Burkill, 1935) and also as treatment for opium addiction in Thailand ( Suwanlert,1975). Mitragynine was isolated in 1907 by D. Hooper and in 1921 by E. Field who gave the alkaloid its name. In 1964, mitragynine structure was successfully determined by D. Zacharias, R. Rosenstein and E. Jeffry. Mitragynine is structurally related to both yohimbe and voacangine. Chemically, mitragynine is 9-methoxy-corynantheidine. Study by Houghton and Ikram (1991) reported that the major alkaloids present in very young leaves of Mitragyna speciosa from Malaysia were shown to be highly conjugated indoles, mitragynaline, corynantheidaline, corynantheidalinic acid and mitragynalinic acidas well as pinoresinol, mitraphylline, mitralactonal, mitrasulgynine and 3,4,5,6-tetradehydromitragynine. Mitragynaline is also present as a minor component in mature leaves of the plant. Although Mitragynine was found in abundance in Malaysian *M. speciosa* , its content was reported to be only about 12% as compared to 66% in Thai *M. speciosa* (Houghton et al., 1991). Other alkaloids contained in *M. speciosa* but in lesser quantity are 7-hidroxymitragynine and mitragynine pseudoindoxyl. The diversity of the alkaloids contained in *M. speciosa* korth harvested from different region calls for further studies and researches, especially in investigating their specific activity, effects and potential applications.

In Malaysia, *M. speciosa* is a controlled plant under poison act (Peraturan-

Peraturan Racun (Bahan Psikotropik), 1989) and its possession without license is illegal

.

Figure 2.1. Mitragyna speciosa Korth plant

(retrieved from Wikipedia on March 20th, 2011)

**2.3. Pharmacology of Opioids**

**2.3.1. Opioid Receptors**

There are four opioid receptor subtypes that have been discovered in the central nervous system (CNS). This is based on the 4 different cDNAs isolated and have been recognized as members of the opioid receptor family. Each of these receptor subtypes exerts its pharmacological response to specific opioid ligands. Opioid receptors belong to seven transmembrane-spinning G protein-coupled receptors (GPCRs) which are activated both by endogenous opioids and exogenous opioids. These receptors are the MOP (mu for morphine) receptor, the KOP (kappa for ketocyclazocine) receptor, the DOP (delta for deferens as it was first identified in mouse vas deferens) and the NOP (

nociceptin , also known as ORL-1 or opioid receptor like ) receptor( Waldhoer et al, 2004). Nociceptin is the latest to be discovered and identified as an endogenous agonist. Antinociception is known to be mainly mediated via stimulation of the mu-, delta-, and kappa- opioid receptor subtypes (Ward, 1983; Yaksh, 1997). Characteristic of opioid receptors and their pharmacologic effects are listed in table 2.1.

The Immunohistochemical studies and in situ hybridization analysis have demonstrated that opioid receptors, especially mu receptors are expressed throughout central nervous system (CNS) which includes amygdala, mesencephalic reticular formation, periaquaductal gray matter (PAG), and rostral ventromedial medulla (RVM) (Mansour et al, 1995). Their activation produces variety of pharmacological effect of opioids, both desirable and undesirable. The locus ceruleus (LC) has been shown to contain both noradrenergic neurons which involves in descending inhibitory pain pathway and also has a high concentrations of opioid receptors and is postulated to play a vital role in perception of alarm, panic, fear and anxiety, which associated with pain perception. This neural activity in LC is inhibited by both exogenous opioids and endogenous opioid peptides. There are studies that propose the existent of subtype of the mu receptor, namely mu1, mu2, and mu3-receptor, although subsequent studies suggest that this difference is the result of post-tanslational modification of the mu receptor itself.

### 2.3.2. Classification of Opioid Compounds

Opioids are classified based on the nature of the synthesis; naturally occurring, semi-synthetic or synthetic. Naturally occurring opioids are morphine, codein,papaverine and thebaine. Semi-synthetic opioids are heroin, dihydromorphone or

morphinone and thebaine derivatives such as buprenorphine. Synthetic opioids are phenylpiperidine series (pethidine,fentanyl, alfentanil, remifentanil an sufentanil), morphinan series( butorphanol, levorphanol), diphenylpropylamine series (methadone) and benzomorphan series (pentazocine).

Table 2.1. Pharmacologic Actions of Opioids and Opioid Receptors in Animal Models (adapted from Miller's Anesthesia, 7th edition)

| | Receptors | Agonist | Antagonist |
|---|---|---|---|
| Analgesia | | | |
| Supraspinal | μ, δ, κ | Analgesic | No effect |
| Spinal | μ, δ, κ | Analgesic | No effect |
| Respiratory Function | μ | Decrease | No effect |
| Gastrointestinal tract | μ, κ | Decrease transit | No effect |
| Psychotomimesis | κ | Increase | No effect |
| Feeding | μ, δ, κ | Increase feeding | Decrease feeding |
| Sedation | μ, κ | Increase | No effect |
| Diuresis | κ | Increase | |
| Hormone secretion | | | |
| Prolactin | μ | Increase release | Decrease release |
| Growth hormone | M, δ | Increase release | Decrease release |
| Neurotransmitter release | | | |
| Acetylcholine | μ | Inhibit | |
| Dopamine | δ | Inhibit | |

### 2.3.3. Intracellular Signal Transduction Mechanism

Since 1990s, the molecular structures and signal transduction mechanisms of the opioid receptors have been studied and established. This analysis of the intracellular signal transduction mechanism upon activation by the opioid receptors has been greatly facilitated by the advancement in cellular and molecular research especially by the expression of the cloned opioid receptors in cultured cells by transfection of the cloned cDNA (Minami et al, 1995). Upon the binding of opioid ligands to the opioid receptors, a series of intracellular reaction occurs which either inhibit or activate certain pathways or signal cascades. Activation of opioid receptor leads to the activation of G-protein, $G_i$ and/or $G_o$, a pertussis toxin (PTX) sensitive G- protein( Law et al, 2000). This will subsequently inhibit adenylate cyclase which then prevent conversion of adenosine triphosphate (ATP) to cyclic adenosine mono phosphate (c-AMP). It also inhibits voltage –gated Calcium channel and as a result it reduces neuronal excitability. On the other hand, it activates the inwardly rectifying potassium ($K^+$) channel and mitogen-activated protein kinase (MAPK) cascade, which results in production of prostaglandins and leukotrines. As a result of these collective intracellular reactions, the cellular neuronal excitability is reduced greatly and thus, no pain transmission is being conducted and perceived in the central nervous system.

### 2.3.4. Analgesic Action of Opioid

Pain control is modulated by various types of peripheral nociceptive receptors and inhibition of the central nervous system response to pain stimulus. The analgesic effects of opioids is exerted by its ability to directly inhibit ascending transmission of

nociceptive information from the spinal cord dorsal horn and activates descending pain pathway that descend from midbrain (for example, periaquaductal gray matter, PAG ), via rostral ventromedial medulla (RVM) to the spinal cord dorsal horn. Thus, opioids produce analgesic effect by direct inhibition at the spinal cord and indirect mediation of descending inhibition pathway.

A significant feature of opioid analgesia is that it is not associated with loss of conscious level. Opioid analgesics are useful in treating nociceptive pain while neuropathic pain rarely responds to them or may require much higher doses of opioid drugs (Romanovsky et al., 1996). While all 4 opioid receptors involved in modulation of pain control, studies showed that the interaction between the mu and kappa receptors may be important for modulating nociceptive transmission at the spinal and supraspinal level.

The level of analgesia produced by an opioid is determined by the intensity of the nociceptive stimulus and the intrinsic efficacy of the opioid. The antinociceptive effect of opioids are enhanced in animal models of inflammation and the sensitization to opioids is secondary to central rather than peripheral site of action.(Klepstad et al, 2000)

## 2.4. Pharmacokinetic and Pharmacodynamic of *M. speciosa* Korth

### Alkaloids

The alkaloid content in *M. speciosa* Korth varies quantitatively from the geographical location and from the different time when the leaves being harvested (Shellard et al., 1974). The alkaloids of *M. speciosa* Korth standardized methanol extract used in this study mainly consist of mitragynine (24.82%), and small quantity of mitraciliatine, corynanthedine and ajmalicine (please refer to GC/MS analysis in appendices, courtesy of Prof. Dr Shariff). This finding was in tandem with the report by

Houghton et al. in 1991 which also showed that mitragynineis the most abundant alkaloids in Malay *M. speciosa* at about 12% (Houghton et al., 1991) and as reported by Kitajima et al in 2002.

### 2.4.1. Mitragynine

Molecular formula for mitragynine is $C_{23}H_{30}N_2O_4$ , its molecular weight is 398.5 g/mol (Jansen & Prast 1988). Physically, mitragynine is a white yellowish amorphous powder, water insoluble, and only soluble in alcohol, chloroform, acetic acid and oily substance (Zacharias et al., 1965).

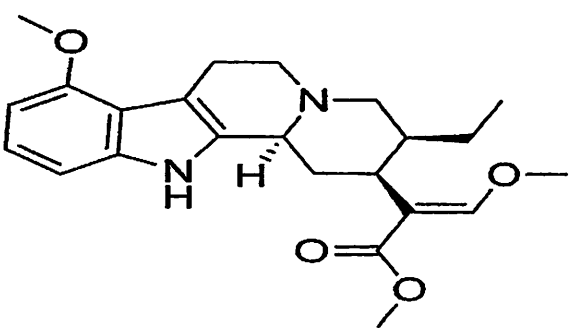

Figure 2.2. Structural formula of mitragynine

(http://wikipedia/mitragynine)

Studies have shown the agonistic action of mitragynine on opioid receptors, in both thermal and mechanically induced nociceptive stimuli in the mice (Matsumoto et al, 1996). Interestingly, subsequent study showed that the selectivity of mitragynine for the supraspinal and spinal opioid receptor subtypes , which is predominantly mediated by mu and delta opioid receptor, differs from that of morphine which is predominantly mu and kappa opioid receptor subtypes (Thongpradichote et al.,1998). In mice, it has been reported that the antinociception mediated by kappa opioid receptor stimulation is depending on the descending serotonergic inhibitory pathway (Vonvoigtlander et al., 1984; Ho et al., 1989). On the other hand, previous study has showed that mitragynine given via intracerebro-ventricle does affect both descending serotonergic and

noradrenergic pathway in mechanical noxious stimuli while thermal noxious stimuli affect descending noradrenergic pathway (Matsumoto et al, 1996).

### 2.4.2. Corynanthedine

This is also known as 9-demethoxy-mitragynine as it lacks the methoxy (MeO) ring at C9, which is characteristic of mitragynine. This indicates corynanthdine has no agonistic activity in guinea pig ileum preparation and lacks analgesic effect similar to mitragynine (Takayama et al., 2002). The study results showed that corynanthedine reversed the morphine-inhibited twitch contraction in guinea pig ileum in a concentration dependent manner and suggest that corynanthedine inhibits the effect of morphine via functional antagonism of opioid receptors. Thus the conclusion is corynanthdine was found to have an opioid antagonistic effect on mu receptors (Takayama et al., 2002).

### 2.4.3. Ajmalicine

Ajmalicine is also found naturally in other plants such as *Rauwolfia* spp. and *Catharanthus* roseus (Wink et al., 1998; Kurt et al., 1981). It is structurally related to yohimbine and act as alpha-1($\alpha$1) adrenergic receptor antagonist, with preferential actions over alpha-2 adrenergic receptors, thus exerting its hypotensive effect rather than hypertensive effects (Roquebert et al., 1984; Wink et al, 1998). It is also a serotonin antagonist and depletes catecholamines from the mouse brain (Neuss, 1980).

### 2.4.4. 7-hydroxymitragynine

7-hydroxymitragynine constitutes about 2% of total alkaloids and exerts analgesic, antitussive, antidiarrheal and primary psychoactive effect.

### 2.4.5. Mitraphylline

Mitraphylline has a vasodilator effect, antihypertensive, muscle relaxer, diuretic, anti-amnesic and possible immunostimulant. It constitutes less than 1% of total alkaloids.

Other active compound contained in the M. speciosa alkaloids constitutes less than 1% of total alkaloids but exert powerful effect than the more abundant active compound. The LD50 (lethal dose 50%) of Mitragyna speciosa korth methanol extract is 4.90 g/kg (Reanmongkol, 2007).

## 2.5. Pharmacokinetic of Morphine

**Absorption** of morphine is influenced by the route of administration. Delivery of the morphine is done via oral in the form of tablets or syrup, intravenous, subcutaneous, intramuscular. Rapid and complete absorption follows parenteral route of administration with peak plasma level usually reached after 20-60 minutes. However, because the hepatic extraction ratio of morphine is high, the bioavailability of oral morphine is significantly lower (20% to 30%) than if administered via intramuscular or subcutaneous injection.

**The distribution** of morphine is determined by lipid solubility, protein binding and degree of ionization. The pKa of morphine is 8.0, which is greater than physiologic pH, which result in only about 10% to 20% of the morphine is in un-ionized form. This