THE EFFECTS OF CHRONIC ADMINISTRATION OF MITRAGYNINE ON CORTICAL OSCILLATIONS IN RATS

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by

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LIST OF SYMBOLS

α	Alpha
δ	Delta
μ	mu
<	Less than
±	Plus, minus
%	Percentage
℃	Degree celcius

LIST OF ABBREVIATIONS

5-HT	5-hydroxytriptamine
ANOVA	Analysis of varience
AP	Antero posterior
CA	Cornu ammonis
COOH	Carboxyl
CPP	Conditioned place preferance
EEG	Electroencephalogram
FCR	Right frontal cortex
FCL	Left frontal cortex
H_2O_2	Hydrogen peroxide
HMG	Hydroxymitragynine
Hz	Hertz
i.p	Intraperitoneal
Kg	Kilogram
LFP	Local field potential
ML	Mediolateral
Mg	Milligram
mm	Millimetre
NAc	Nucleus accumbens
PFC	Prefrontal cortex
SEM	Standard error of mean
VTA	Ventral tegmental area

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KESAN – KESAN PEMBERIAN KRONIK MITRAGYNINE TERHADAP KUASA OKSILATORI KORTIKAL DALAM TIKUS

ABSTRAK

Mitragynine adalah sebatian alkaloid utama daripada daun *M. speciosa* Korth. Daunnya digunakan secara meluas untuk menghilangkan rasa sakit serta membantu dalam mengurangkan kesan gejala tarikan. Kajian terdahulu telah merekodkan bahawa bahan ini memang mempengaruhi mekanisma kognitif pada manusia namun perdebatan mengenai potensi kesan sampingan bahan termasuk risiko ketagihan dan penurunan kognitif masih berterusan dan juga aktiviti saraf asasnya masih tidak jelas. Oleh itu, kajian ini dirancang untuk menyiasat perubahan dalam kuasa spektrum otak dan koheren theta selepas pendedahan berulang kepada mitragynine dalam tikus yang bergerak bebas. Tikus Sprague-Dawley jantan diimplan dengan elektrod pada korteks hadapan kanan dan kiri, hippocampal cornu ammonis (CA1), subiculum dan korteks deria untuk rakaman EEG tanpa wayar. Mitragynine (1, 5, dan 10 mg/kg) diberikan setiap hari selama 28 hari, dan aktiviti EEG direkodkan pada hari 7,14,21 dan 28. Bacaan dihantar ke penerima dan dianalisis untuk julat frekuensi yang berbeza, delta (0.1- 4Hz), theta (4-7Hz), alfa (7-13Hz) dan beta (13-30Hz). Hasilnya telah menunjukkan perubahan spesifik frekuensi dalam kuasa spektrum yang berlaku secara selektif di kedua-dua kawasan kortikal dan hippocampal. Di kawasan kortikal, peningkatan umum dalam kuasa beta dengan pengurangan cagaran dalam kuasa alpha dilihat sebagai kesan baru mitragynine, manakala pengurangan kuasa delta dihargai di Kawasan kortikal dan hippocampal. Pengurangan akibat mitragynine dalam koherensi theta (4-7Hz) dilihat sebagai gangguan dalam hubungan fungsi antara korteks hadapan kiri dan korteks deria. Dapat disimpulkan bahawa, penemuan ini menunjukkan perubahan spesifik frekuensi yang disebabkan oleh mitragynine dalam aktiviti

oksilatori saraf kortikal dan koheren theta yang ditindas boleh berpotensi memberi kesan kepada fungsi kognitif.

THE EFFECTS OF CHRONIC ADMINISTRATION OF MITRAGYNINE ON CORTICAL OSCILLATIONS IN RATS

ABSTRACT

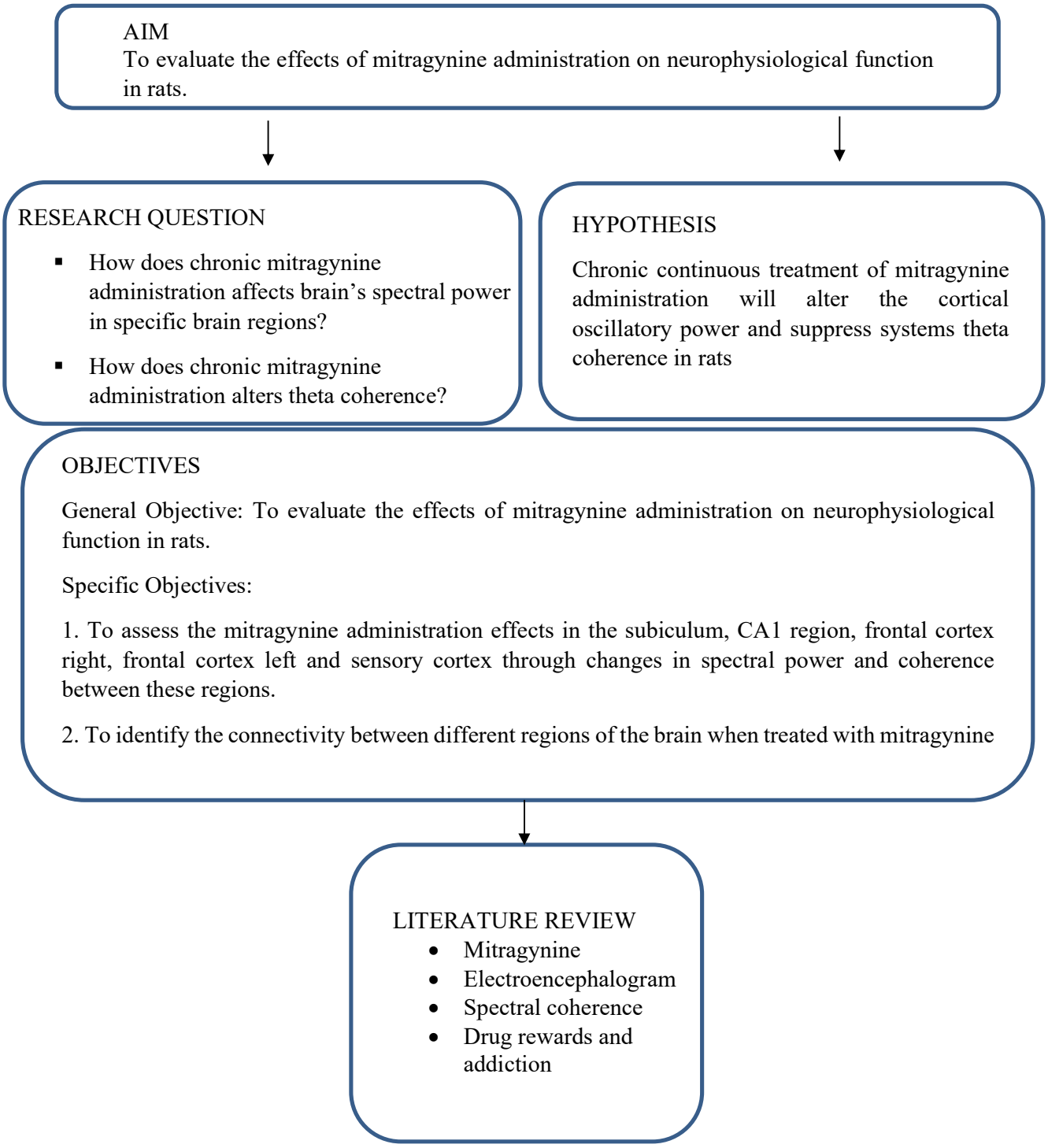
Mitragynine is the significant alkaloid extracted from the leaves of *M. speciosa* (Kratom). The leaves have been extensively used to relieve pain as well as to aid in opioid withdrawal symptoms. Previous studies have recorded that drug do affect cognitive mechanisms in human yet the debate over the potential side effects of the drug which includes addiction risk and cognitive decline is still ongoing and also its underlying neural activity remains unclear. Thus, this study was designed to investigate the changes in brain spectral power and theta coherence after repeated exposure to mitragynine in freely moving rats. Male Sprague-Dawley rats was implanted with electrodes on right and left frontal cortex, hippocampal cornu ammonis (CA1), subiculum and sensory cortex for wireless EEG recording. Mitragynine (1, 5, and 10 mg/kg) was administered for 28 days, and EEG activity was recorded on day 7,14,21 and 28. The readings was transmitted to receiver and analyzed for different frequency range, delta (0.1-4Hz), theta (4-7Hz), alpha (7-13Hz) and beta (13-30Hz). The results have demonstrated frequency specific changes in spectral power occurring selectively in both cortical and hippocampal regions. In cortical regions, a general increase in beta power with collateral reduction in alpha power is seen as novel effects of mitragynine, while a reduction in delta power is appreciated in both cortical and hippocampal regions. Mitragynine induced reduction in theta coherance (4-7Hz) was seen as disruptions in functional connectivity between left frontal cortex and sensory cortex. It can be concluded that, these findings show mitragynine induced frequency-

specific changes in cortical neural oscillatory activity and suppressed theta coherence could potentially impact cognitive functioning.

CHAPTER 1

INTRODUCTION

1.1 Conceptual Framework



1.2 General Introduction

It is an interesting fact that people are engaging in using substances to enhance their abilities, some unaware of the risks. An example of this kind of behavior is an increased intake, which is usually repeated due to pleasurable feelings inspired by the rewarding substance. Kratom is one of the new trendy examples of these drugs. Reducing pain from withdrawal symptoms is relieved widest of the reasons to ingest Kratom include lowering opium addiction, opiate substitution, and reducing pain from withdrawal symptoms (Chien et al.,2017). But sad to say, kratom does also have some deleterious effects when used for a prolonged time (Singh et al., 2019). This is an excellent reason showing that attention is needed to give for this drug. Everyone should be aware that Kratom as a botanical substance not only carries a risk to public health but also potential for abuse. However, providing health care for kratom users has been challenging to the authorities due to difficulties in identifying them as there is no convenient test for mitragynine detection (Kruegel & Grundmann,2017). Thus, through these knowledge points, we understand mitragynine better, whether as a therapeutic agent or as a harmful agent.

Mitragynine, an alkaloid extracted from the leaves of *M. speciosa* (Kratom), has been used broadly to ease pain especially in opioid withdrawal symptoms. This has been shown to bind in some degree to several non-opioid CNS receptors, including alpha-2 adrenergic receptor (α2R), adenosine A2a, dopamine D2, and the serotonin receptors 5-HT2C and 5-HT7 Still, there is no report on the strength of these affinities (Kruegel et al., 2016).

Dopamine neurotransmitter system's activity act as a path for most addictive drugs to produce their effects where modulation of high gamma oscillatory activities

seen in the striatum and nucleus accumbens can be associated with neural processing as motor component of a reward function (Reakkamnuan et al.,2017).

Dependance and tolerance with prolonged consumption of this substance have been discussed in human study while termination is seen to cause series of aversive withdrawal symptoms (Suhaimi et al.,2016). Thus, higher dosage of Mitragynine consumption is suggested to achieve the desired effects (Hassan et al., 2013). At the same time, withdrawal symptoms include hostility, aggression, aching of muscles and bones, jerky movements of the limbs, anorexia, weight loss, insomnia, and psychosis (Suhaimi et al.,2016).

In humans, drug abuse and addiction are associated with chronic consumption of *M. speciosa* preparations. Mirtagynine has also been studied to give rise to rewarding effects by itself (Yusoff et al., 2016) while on the other hand also intensifies punishment resistance in natural reward-seeking (Ismail et al., 2017). The pleasure of reward is led by activation of the mesolimbic dopaminergic pathway. Several areas in the brain such as Ventral Tegmental Area (VTA), Nucleus Accumbens (NAc), amygdala, hippocampus, and the Prefrontal Cortex (PFC) makes up the reward pathway (Ismail et al.,2017). There is also study that indicate mitragynine as not rewarding but at high dose it might be aversive while in the same study it does mention that 7- hydroxymitragynine has impaired reward function (Behnood-Rod et al.,2020).

Moreover, there is data strongly suggesting the pharmacological mechanism of mitragynine is not the same as other prototypical opioids even though there is evidence of binding affinity of the drug at opioid receptors (Hiranita et al.,2019). One study has also reported that mitragynine produce a stronger therapeutic effect in combination with other effective opioid agonists and can be used as an alternative treatment. This also shows that central nervous system actions of mitragynine is not the same as other

opiates according to local field potential patterns and levels of spontaneous motor activity (Hassan et al.,2019). In contrast to this, a study shows mitragynine discontinuation is not associated with overt withdrawal effects and suggests it can be used to treat opioid withdrawal symptoms and opioid use disorders as potential therapeutic drugs (Harun et al.,2020). Damages in passive avoidance learning and memory consolidation and retrieval by acute mitragynine have been reported perhaps mediated by a disruption of cortical oscillatory activity, including suppressing low-frequency rhythms (delta and theta) in the electrocorticogram (Yusoff et al.,2014). On the other hand, impairment in passive avoidance and object recognition learning was seen with chronic mitragynine treatment (Yusoff et al.,2014). Therefore, mitragynine can be proposed in classification as harmful drug where these findings gives insight for the drug as an addiction potential with cognitive impairments.

Through the text, it is known that previous studies have recorded that Mitragynine do affect cognitive mechanisms in human yet the debate over the potential side effects of the drug which includes addition risk and cognitive decline is still ongoing and also its underlying neural activity remains unclear. Thus, this study was aimed to evaluate the effects of mitragynine administration on neurophysiological function in rats. This study was designed to investigate the changes in brain spectral power and theta coherence after repeated exposure to mitragynine in freely moving rats. Male Sprague-Dawley rats were used and implanted with electrodes over brain regions for EEG recording. Mitragynine (1, 5, and 10 mg/kg) was administered intraperitoneally daily for 28 days, and brain activity between these regions of interest was recorded on days 7, 14, 21, and 28.

Impairment between spatial learning and memory was also discovered with chronic mitragynine administration, where learning deficit was observed as that

chronic morphine would induce (Ismail et al.,2017). There is also a study by Hassan and colleague in 2019, demonstrating intense impairments in excitatory synaptic transmission and spatial memory acquisition in the hippocampal CA1 field as well as an average suppression of long-term potentiation and also shows that in rodents' potential cognitive dysfunction and process disruption in hippocampal formation is caused by mitragynine at higher doses.

In cognition, drug-induced changes are frequently related with brain activity alteration whereby the net effects or brain's balance of neurotransmission is reflected with brain field potential within a respective frequency range (delta, theta, alpha and beta powers). After drug administration, changes in EEG pattern are acknowledged as EEG fingerprints or biomarkers that changes recognized fingerprints like those induced by antidepressants (Cheaha et al.,2015). Studies involving rats, have reported that theta wave rhythmicity observed in hippocampus easily as well as can be detected in few other cortical and subcortical brain structures. In terms of magnitude and frequency range, treatment with mitragynine also produces slow-wave activity suppression which is a similar action as following treatment with fluoxetine (Cheaha et al.,2015). As well the power of gamma oscillational has also been seen to prove the effectiveness of mitragynine for the treatment of ethanol withdrawal symptoms (Cheaha et al.,2015).

1.3 Rationale of study

The potential therapeutic properties of mitragynine versus the addictive consequences of mitragynine have been a source of discussion. The goal of this research is to learn more about how the medicine affects the brain's function in various areas. Mitragynine's effects on cortical oscillations are significant, indicating that it may have an impact on cognitive functioning. Furthermore, it provides a greater opportunity to comprehend the nature of the substance mitragynine and its potential effects on the human brain. Furthermore, this trial will assist us in linking drug intake to cognitive impairment via changes in brain activity. This will be used as supporting evidence in any future medication and cognitive decline studies. When kratom is consumed in high doses or over time, it affects cognitive function and can be used to detect the addicted patient's cognitive decline earlier. As a result, this can both advise and educate them in seeking expert aid. Understanding the effects of mitragynine on human brain activity will aid doctors, neurologists, and other health care professionals in assisting the addicted individual in recovering and thereby improving their quality of life. As a result, the negative impacts on the patient's personal and social life will be limited.

1.4 Objectives and Hypothesis

GENERAL OBJECTIVE

To evaluate the effects of mitragynine administration on neurophysiological function in rats.

SPECIFIC OBJECTIVE

1. To assess the mitragynine administration effects in the subiculum, CA1 region, right frontal cortex, left frontal cortex and sensory cortex through spectral power and coherence changes between these regions.
2. To identify the connectivity between different regions of the brain when treated with mitragynine.

RESEARCH QUESTION

1. How does chronic mitragynine administration affects brain's spectral power in specific brain regions?
2. How does chronic mitragynine administration alters theta coherence?

HYPOTHESIS

Chronic continuous treatment of mitragynine administration will alter the cortical oscillatory power and suppress systems theta coherence in rats

CHAPTER 2

LITERATURE REVIEW

2.1 The drug – Kratom

M. speciosa Korth *(M. speciosa)* or Kratom is a medicinal herb of the Rubiaceae (coffee) family that can be easily found in tropical and sub-tropical regions of Asia (Hassan et al.,2013). It is found as a native plant in Philippine islands, New Guinea, and Southeast Asia, predominantly Malaysia, Thailand, and Indonesia. Kratom's major alkaloid, mitragynine has been criminalized by Malaysian government due to the rising concerns over the plant's narcotic properties and abuse liabilities, under the Third Schedule of Poisons (Psychotropic Substances) Regulations, Poison Act 1952 (Veltri & Grundmann.,2019). Recreational use and abuse of kratom still prevalent in Malaysia and Thailand despite legal restrictions in certain countries (Singh et al., 2019; Singh et al., 2017; Ahmad and Aziz, 2012). Western users got attracted to use of kratom to self-medicate for opioid withdrawal and chronic pain with the claims and reports of its potential as cheap opioid substitute. In recent decades, it is also being sold as a dietary supplement in United Stated and Europe (Müller et al., 2020; Coe et al., 2019; Grundmann, 2017).

Two types of kratom can be identified based on the colour of the leaf vein, which can be either green or red while the red vein is usually preferred as it is characterized for its bitterness and longer effects (Adkins et al.,2011). The fresh leaves are mostly used either chewed swallowed or as a powder at a dosage of normally 10 to 30 fresh leaves per day, but they can also be dried for smoking or to make tea (Tanguay,2011). Kratom’s pharmacokinetics in humans has not been well studied, however studies in rats have showed that despite with higher dose oral administration presents with poor

bioavailability compared to intravenous administration. Smaller fraction of absorption may cause this reduced oral bioavailability due to poor aqueous solubility of *M. speciosa* (Parthasarathy et al.,2010). A highly solubilized and ionized basic drug, in the stomach, Mitragynine, thus reduces its absorption and therefore bioavailability (Ramanathan.S et al.,2015). Additionally, when using Caco-2 cells mitragynine showed better permeability than 7-HMG to predict intestinal absorption (Manda et al.,2014).

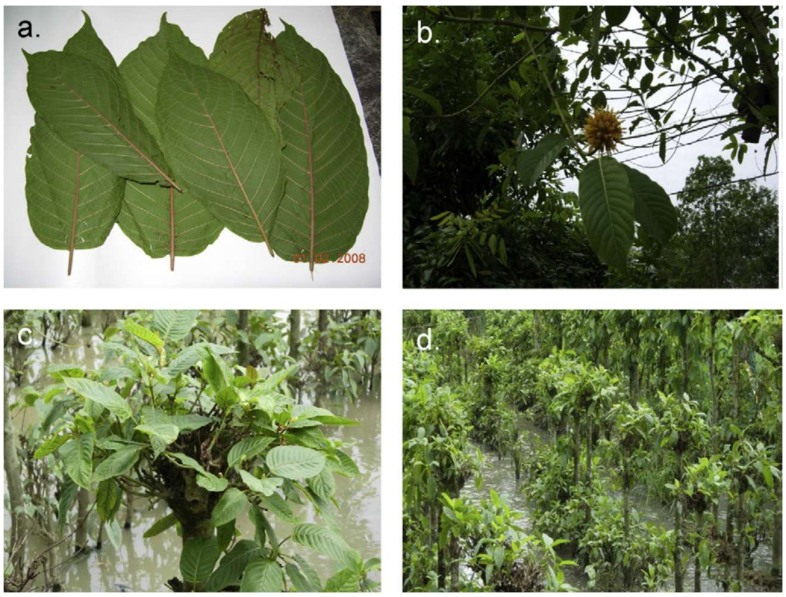


Figure 2.1: The plant M. speciosa Korth. (a) Leaves of the plant, (b) naturally occurring trees, (c and d) cultivated plants (adapted from Hassan et al.,2013).

Its antinociceptive and psychostimulant effects as well as anti-inflammatory and muscle relaxant properties is well recognized as a local medicinal plant (Hassan et al., 2013; Chien et al.,2017; Raffa, 2014). The effects of kratom are dose-dependent where it produces stimulant effects at a low dose and sedative-narcotic effects at higher dose (Meepong & Sooksawate, 2019). Claims of being energized, light-headed, alert, relaxed, contented and sedated by kratom users have been reported by Ahmad and Aziz

(2012). All these effects usually last between 1 to 6 hours. Gratifying impact and useful effort in reducing the morphine and ethanol withdrawal effects has been proven with *Mitragyna. speciosa*.

More than 40 alkaloids have been identified from the Kratom extract (Meireles et al.,2019). Mitragynine, is the primary active alkaloid isolated from Kratom. This constituent with similar effects to morphine has been identified as a partial opioid receptor agonist. Other active alkaloids include 7-hydroxymitragynine (HMG), speciociliatine, speciogynine and paynantheine (Meireles.2019).

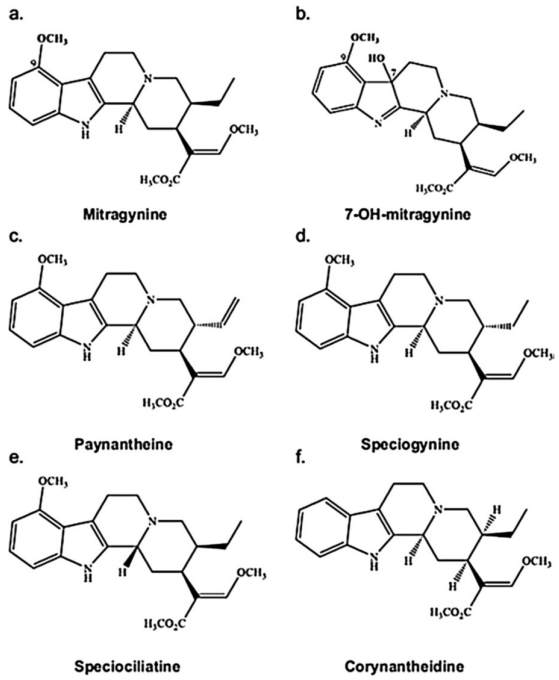


Figure 2.2. Chemical structure of mitragynine and its major analogues (adapted from Hassan et al., 2013).

Several studies have revealed the high binding affinity for supraspinal mu(μ) and delta(δ) opioid receptors for kratom's major alkaloid, mitragynine, and also for the less abundant constituent, 7-hydroxymitragynine, which controls the antinociceptive and antitussive actions and their rewarding properties while central opioid receptors are more relevant for other psychoactive effects (Yusoff et al., 2017; Matsumoto et al.,

2006). A study has shown that mitragynine has different affinity to different opioid receptor subtypes. Highest affinity to kappa opioid receptors is seen towards mitragynine and then followed by mu and delta opioid receptors. Differences in interaction between polar structures of mitragynine can be the reason for these differences in binding affinity where location at the membrane of a set of N-termini and carboxyl (COOH) transmembrane 4 and extracellular loop 2 and 3 differentiates between opioid receptors which are mu, kappa and delta receptors (Taufik Hidayat et al., 2010).

Opioids are one of the highly harmful types of drugs, it has to be well understood that we are still in need of a better substitute pharmacotherapy for opioid agonist replacement therapy as well as other substance use disorders. Moreover, specifically for the management of chronic pain as well as opioid withdrawal, there is a severe need to develop safer therapeutic alternatives to opioids. Although they have activity at the opioid receptors, the active ingredients in Kratom are not opioid compounds.

Mitragynine (kratom) has been studied to bind in some degree to several non-opioid CNS receptors, including adenosine A2a, dopamine D2, alpha-2 adrenergic receptor (α2R) and the serotonin receptors 5-HT2C and 5-HT7, but the strength of these affinities has not been reported (Boyer et al., 2008). The inhibitory effect of mitragynine against apomorphine mediated by its interaction with the dopaminergic system has suggested the antipsychotic-like effect of mitragynine (Vijeepallam et al.,2016). Studies in humans have unfold that prolonged consumption of this plant will lead to dependence and tolerance while termination caused a series of aversive withdrawal symptoms (Suhaimi et al.,2016). Thus, the increasing dosage is needed to achieve the desired effects (Hassan et al., 2013). Meanwhile, withdrawal symptoms include jerky movements of the limbs, hostility, aggression, weight loss, insomnia, aching of muscles

and bones, anorexia and psychosis (Suhaimi et al.,2016). In humans, chronic consumption of *Mitragynine. speciosa* preparations is associated with drug abuse and addiction. This drug not only prompts rewarding effects by itself (Yusoff et al., 2014) but also intensifies punishment resistance in natural reward-seeking (Ismail et al., 2017).

Acute mitragynine independently damages, memory consolidation and retrieval as well as passive avoidance learning including suppressing low-frequency rhythms (delta and theta) in the electrocorticogram perhaps mediated by a disruption of cortical oscillatory activity (Yusoff et al.,2014). Previous studies have reported that mitragynine have caused a deficit in learning and memory functions and also impaired behavioral performances (Yusof et al.,2016; Hassan et al.,2019; Singh et al.,2019; Ismail et al.,2017). Chronic mitragynine treatment led to impaired passive avoidance and object recognition learning. Therefore, mitragynine can be classified as a harmful drug where these findings give evidence for its addiction potential with cognitive impairments. Drug-induced changes in cognition are frequently followed by the alteration of brain activity whereby the brain field potential within a respective frequency range (delta, theta, alpha and beta powers) reflect the net effects or balance of neurotransmission in the brain (Dimpfel,2008).

However, these studies have yet to prove the exact neural mechanism as well drug dose dependent changes in brain region which then lead to cognitive changes. Thus, to further understand this mitragynine-induced cognitive impairment, this study aimed to learn the changes in spectral power induced by chronic mitragynine treatment by recording brain EEG activity in several brain regions namely right and left frontal cortex, CA1(cornu ammonis), subiculum and sensory cortex in Sprague-Dawley rats. These regions were chosen based their roles in the learning and memory processes. The