
**UNIVERSITI PUTRA MALAYSIA**

# *INVESTIGATING THE EFFECT OF MITRAGYNA SPECIOSA METHANOL EXTRACTION ON REWARDING PATHWAY IN BRAIN OF SPRAGUE DAWLEY RAT*

**NURUL AQILAH BINTI AHAT @ SALIM**

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**THESIS SUBMITTED TO DEPARTMENT OF BIOMEDICAL SCIENCE, FACULTY OF MEDICINE AND HEALTH SCIENCES, UNIVERSITI PUTRA MALAYSIA, IN FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR SCIENCES (BIOMEDICAL SCIENCE)**

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## ABSTRACT

## INVESTIGATING THE EFFECT OF *MITRAGYNA SPECIOSA* METHANOL EXTRACTION ON REWARDING PATHWAY IN BRAIN OF SPRAGUE DAWLEY RAT



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**Introduction:** *Mitragyna speciosa*, which is also commonly known as kratom in Thailand and ketum in Malaysia, is a native plant to Southeast Asia region especially, Thailand and Malaysia. This plant is used as to treat injury or illness and also it is known for its analgesic uses. Previous study had found that this *Mitragyna speciosa* has effect on brain which leads to hedonic consequences (reward). Dopamine is released from ventral tegmental area (VTA) and then being transmitted to ventral striatum. Increase in dopamine may lead to an addiction of herbs or drugs that caused such consequences. **Objectives:** This study generally aims to investigate the effect of *Mitragyna speciosa* methanol extraction on rewarding pathway in Sprague Dawley rat brain. **Hypothesis:** This *Mitragyna speciosa* methanol extraction will lead to increment of dopamine in rat brain, particularly on rewarding pathway in the rat brain for both acute and chronic administration of the extracts. **Methodology:** Extracted *M. speciosa* will be administered to two big groups of rat which are first group for sub-acute study and second group is for chronic study. In each group, there would be 5 sub-groups which are administration of positive control, negative control, low, medium and high concentration of extracted *M.speciosa*. The period of sub-acute study is up to seven days and for chronic study it is up to twenty one days. **Results:** The increment of dopamine level in striatum and prefrontal cortex can be seen clearly after the administration of *M. speciosa* extracts. **Conclusion:** *Mitragyna speciosa* does affect the rat brain particularly in rewarding pathway in which more dopamine is released in the brain to lead to hedonic consequences.



*Keywords: Mitragyna speciosa,* rewards, rat brain, dopamine, ketum, kratom

## ABSTRAK

# MENYIASAT KESAN EKSTRAK METANOL *MITRAGYNA SPECIOSA* TERHADAP LALUAN GANJARAN DALAM OTAK TIKUS SPRAGUE DAWLEY



**Nurul Aqilah Ahat@Salim[1], Mohamad Aris Mohd Moklas[2], Tengku Azam Shah Tengku Mohamad[1]**

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**Pengenalan:** *Mitragyna speciosa,* yang juga dikenali sebagai kratom dalam bahasa Thai dan ketum dalam bahasa Melayu, adalah tanaman asli kepada rantau Asia Tenggara, terutama sekali Thailand dan Malaysia. Tanaman ini digunakan untuk merawat kecederaan dan penyakit, dan ia juga terkenal untuk kegunaan analgesiknya. Kajian terdahulu mendapati bahawa spesifikasi Mitragyna ini memberi kesan pada otak yang membawa kepada kesan hedonik (ganjaran). Dopamin dilepaskan dari kawasan tegmental ventral (VTA) dan kemudian dihantar ke striatum ventral. Peningkatan dopamin boleh menyebabkan ketagihan kepada herba atau ubat-ubatan yang mengakibatkan kesan tersebut. **Objektif:** Kajian ini secara amnya bertujuan untuk mengkaji kesan pengekstrakan metanol Mitragyna speciosa pada laluan ganjaran di otak tikus Sprague Dawley. **Hipotesis:** Ekstrak metanol *Mitragyna speciosa* ini akan menyebabkan peningkatan dopamin dalam otak tikus, terutama pada laluan ganjaran di otak tikus untuk kedua-dua akut dan kronik. **Metodologi:** *M. speciosa* yang diekstrak akan diberikan kepada dua kumpulan tikus besar yang merupakan kumpulan pertama untuk kajian sub-akut dan kumpulan kedua adalah untuk kajian kronik. Dalam setiap kumpulan, akan terdapat 5 subkumpulan yang merupakan kawalan positif, kawalan negatif, kepekatan M.speciosa yang diekstrak rendah, sederhana dan tinggi. Tempoh kajian sub-akut adalah hingga tujuh hari dan untuk kajian kronik adalah sehingga dua puluh satu hari. **Hasilan:** Peningkatan tahap dopamin dalam striatum dan korteks prefrontal dapat dilihat dengan jelas setelah pemberian ekstrak M. speciosa. **Konklusi:** *Mitragyna speciosa* mempengaruhi otak tikus terutamanya dalam jalan yang bermanfaat di mana lebih banyak dopamin dilepaskan di otak untuk membawa kepada kesan hedonik.



*Kata kunci: Mitragyna speciosa,* ganjaran, otak tikus, dopamine, ketum, kratom

## ACKNOWLEDGEMENT

Alhamdulillah, all praises to the Almighty Allah *Subhanahu Wa Ta'ala,* for giving me strength, patience, motivation, inspiration and a good health condition throughout my FYP journey until the completion of thesis. This journey would not have been completed without the support from many people.

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# CHAPTER 1

# INTRODUCTION

## 1.1 Study Background

Plants have always been used for therapeutic effects ever since ancient times ago as it can be found abundantly around the world in which it is usually called as medicinal plants. There are about 2000 medicinal plants in Malaysia that was being reported that have benefits on health (Latif, A., 1985). Not to forget about worldwide, according to WHO, about 80% of world population is being dependent on these medicinal plants in order to stay healthy. These medicinal plants are then being used as a framework in scientific studies in order to design new drugs as different plants can contribute to different health benefits properties. These continuous researches on most of medicinal plants might make a breakthrough in drug discovery as those medicinal plants offer valuable information to be used in treatment of an injury or disease.

*Mitragyna speciosa*, which is also commonly known as kratom (ketum in Malay), is a tropical tree which is native in Southeast Asia region. The tree is indigenous particularly to Thailand, Malaysia, Indonesia and other tropical countries. People of Southeast Asian, particularly in southern Thailand and northern Malaysia, traditionally used the plants, particularly its leaves, as a treatment for fever, pain and other diseases. This plant is also used to improve fatigue condition, have better tolerance towards heat and sunlight and also enhance physical endurance (Sirivongs Na Ayudhya and Assanangkornchai, 2005, Vicknasingam et al., 2010). These findings of kratom piqued scientists'

curiosity thus there are numerous studies have been done in order to determine the role of *Mitragyna speciosa* and also its effects on health, cognitive behavioural, psychological and social impact (Ahmad and Aziz, 2012, Apryani et al., 2010).

Leaves of *Mitragyna speciosa* contains a compound, mitragynine, that can act on the brain in which can result in hedonic consequences (which is also known as reward). It has been found that dopamine is required component of rewards (Arias-Carrión and Pŏppel, 2007, Phillips et al., 2008). This dopamine (DA) originates from ventral tegmental area (VTA), in which this area is the one who responsible to release dopamine. These neurons which associated with VTA-DA are necessary components in rewarding system in brain (Dafny and Rosenfield, 2016). This reward pathway is mostly known as mesolimbic pathway (which is also known as mesolimbic dopamine system) (Dreyer, J. L., 2010). This pathway joins VTA to ventral striatum located in basal ganglia in the forebrain. The ventral striatum is consists of nucleus accumbens and the olfactory tubercle (Ikemoto, S., 2010). Hence, this study is being carried out in order to find out the effect of *Mitragyna speciosa* on reward pathway on the brain, particularly on Sprague Dawley rat brain.

## 1.2 Problem Statement

It has been known that *Mitragyna speciosa* can affect brain, particularly in rewarding pathway in which dopamine and its metabolite is involved. This *Mitragyna speciosa* can affect brain on how much dopamine and its metabolite being released in the brain. However, approximation of dopamine and its metabolite released in the brain is not distinctly known.

## 1.3 Objectives

### 1.3.1 *General Objectives*

General objective for this study is to investigate the effect of *Mitragyna speciosa* methanol extraction on rewarding pathway in brain of Sprague Dawley rat.

### 1.3.2 *Specific Objectives*

- To measure the dopamine, serotonin and its metabolite level in rat brain after acute administration of *Mitragyna speciosa* methanol extraction by using HPLC.
- To measure the dopamine, serotonin and its metabolite level in rat brain after chronic administration of *Mitragyna speciosa* methanol extraction by using HPLC

## 1.4 Hypotheses

*Mitragyna speciosa* methanol extraction will exhibit signififcant effect in rat brain particularly the level of dopamine, serotonin and its metabolite in both acute and chronic administration of the extracts.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 The Overview of *Mitragyna speciosa*

*Mitragyna speciosa* Korth is belonging to *Rubiaceae* family which is also known as coffee family. This *M. speciosa* has many different common names used to call the plants, particularly to call its leaves such as ketum, kratom and biak-biak, particularly in Thailand and Malaysia (Adkins et al., 2011). This plant is native to Southeast Asia region; however, the plant is also cultivated somewhere else apart from being cultivated in Southeast Asia only. This kratom's tree commonly can be found at south part of Thailand and it seems to be easily obtained from teashops in which it is used as a replacement of opium and alcohol (Meireles et al., 2019). Apparently, there are two types of kratom can be classified according to its leaves colour, in which there are two colours, green and red. Thailand native prefer the red one as it more bitter and can last longer compared to green one (Adkins et al., 2011).

*Figure 1: Red vein of kratom plant.*

*Figure 2: Green vein of kratom plant*

This plant traditionally has been used since ages ago due to its health benefits properties either the leaves is chewed, smoked, made it into tea or ingested tar-like solid of the plant that has been extracted. The leaves are used in order to enhance physical endurance, or even as a substitute of opium. *M. speciosa* also can be used for chronic period to treat anorexia, weight loss, constipation and hyperpigmentation of the face (Adkins et al., 2011). Apart from that, kratom was used as its exhibit medicinal values such as treating fever, diarrhea, diabetes and pain (Meireles et al., 2019). Although it has medicinal values, obtaining kratom is actually illegal in Thailand and also this kratom is being banned in Malaysia due to problem in youth being addicted to the leaves of kratom (Adkins et al, 2011).

## 2.2 Chemical Constituents of *Mitragyna speciosa*

According to Takayama (2004), *M. speciosa* plant that is originated both from Thailand and Malaysia has different chemical constituents. For Thailand plant of *M. speciosa,* the major constituents found is mitragynine which comprises of 66.2% from the crude base. It has been also been found in conjunction with its analogues which are speciogynine (0.8%), speciociliatine (0.8%) and paynantheine (8.6%). New alkaloid, 7α-hydroxy-7H-mitragynine, (refer Figure 1) a transformation of mitragynine was also isolated in which it is considered as minor constituents found in the *M. speciosa* (Takayama, 2004).

*M. speciosa* plant from Malaysia consists of similar alkaloids with Thai plant in which mitragynine, speciogynine, speciociliatine, paynantheine and 7-hydroxymitragynine are isolated. Mitragynine is still the major constituent in the plant, however, the yield of mitragynine in Malaysian plant compared to Thai plant is quite lower in which estimated only 12% mitragynine of alkaloids extracts. Additionally, there are two more minor constituent that were isolated as well which are mitragynaline and pinoresinol (refer figure 2). Along with it, three new indole alkaloids were also obtained in the plant which are mitralactional, mitrasulgynine and 3,4,5,6-tetradehydromitragynine (Takayama, 2004).

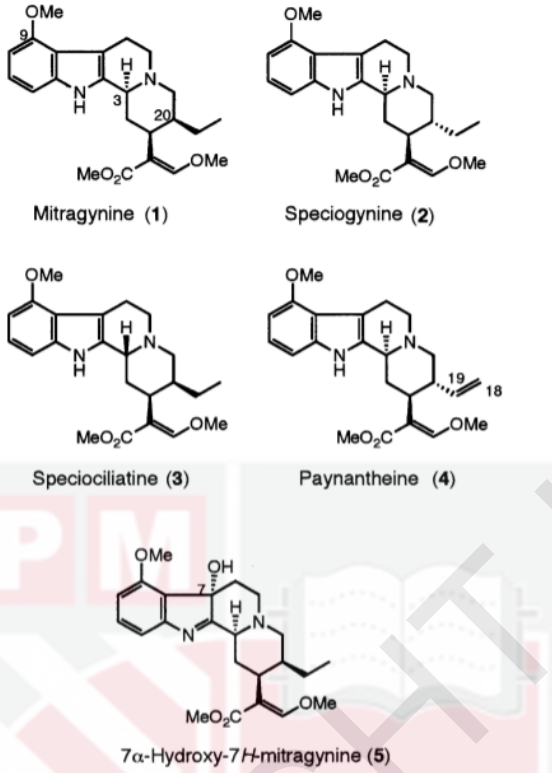


*Figure 3: Major constituents of Mitragyna speciosa (*Adapted from: *Takayama et al., 2004*)

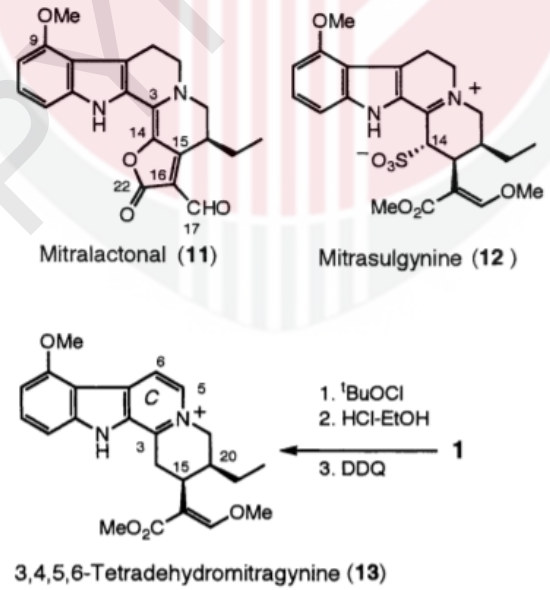


*Figure 4: Minor constituents of Mitragynine speciosa* (Adapted from: *Takayama et al., 2004*)

## 2.3 Mitragynine

The key compound that is important to this research is mitragynine which is the major constituent of *M. speciosa*. Being a replacement for opium as mentioned above shows that this plant especially acts on the brain. This is due to mitragynine have opioid-like effects in which make there is a suggestion saying that it is estimated 13 times more potent compared to morphine. Kratom can acts as a stimulant or have opioid-like effect. It still depends on the dosage of Kratom consumed which the affects range from levels of stimulation/mood improvements and sedation/analgesia (Warner et al., 2015).

Mitragynine is μ-opioid subtype receptors agonists and it exhibits activity on suprasinal μ- and δ-opioid receptors which leads to the analgesic effects of Kratom. Studies suggested that the analgesic effects are caused by inhibition of neurotransmitter from being released at the nerve endings of vas deferens. Moreover, its acts as stimulant is due to the stimulation of serotonergic 5-$HT_{2A}$ receptors and postsynaptic alpha-2 adrenergic receptors is being blocked (Warner et al., 2015).

However, up till now, there is still lacking in studies done on the addiction properties of the plant especially on analysis of neurochemical in brain after administration of this plant. Thus, with this study, we specifically want to look at the effect of *Mitragyna speciosa* especially mitragynine rewarding pathway in the rat brain.

## 2.4 Taxonomy of Mitragyna speciosa

***Table 1: The taxonomical classification of Mitragyna speciosa***

| | |
|---|---|
| **Scientific Name:** | *Mitragyna speciosa* |
| **Vernacular Name:** | Kratom or Ketum |
| **Kingdom:** | Plantae |
| **Division:** | Tracheophytes |
| **Order:** | Gentianales |
| **Genus:** | Rubiceae |
| **Family:** | *Mitragyna* |

## 2.5 Rewards and Motivation

According to Cook and Artino, 2016, motivation is defined as the process in which goal-directed task are initiated and maintained. Usually, motivation is the source of energizing behaviour and will power in order to achieve goal or to even get something completed (Simpson & Balsam, 2016). It acts as a driving force for an organism to adapt and survive in changes of environment whether internally or externally.

Having reinforcement in order to complete a task usually would motivate the organism to do better or enhance their productivity, especially positive reinforcement. It usually would excite them knowing there is something good waiting for them if they do better in their task. Reinforcement is defined as a process to increase the desired behaviour probability in which it will be delivered at the end of the desired behaviour (Gordan & Krishanan, 2014). Reinforcement can either be a compliment or a physical reward to be given to the individuals who successfully perform a desired behaviour.

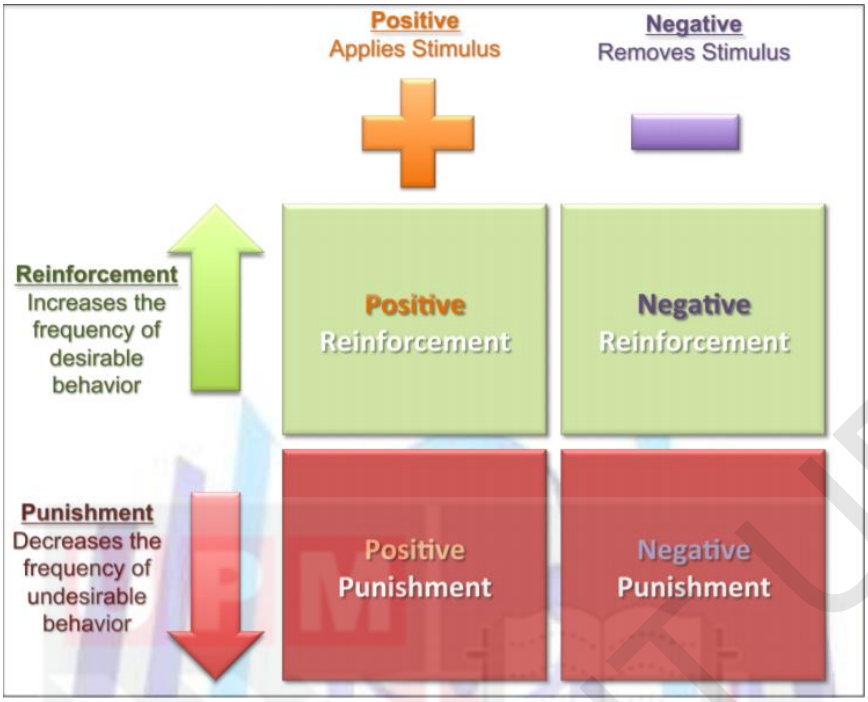


*Figure 5: Reinforcement* (Adapted from: *Gordan & Krishanan, 2014*)

There are two types of reinforcement which includes positive reinforcement, negative reinforcement and punishment. Positive reinforcement is when individual being rewarded when keeping up a desired behaviour. The reward either can be compliment or physical reward. Meanwhile, negative reinforcement is when an individual can avoid undesired stimulus when they keep up the desired behaviour. Frequently, negative reinforcement being mistaken with punishment. Punishment is when a desired or pleasant stimulus or reward is being removed in order to decrease the probability of undesired behaviour (Gordan & Krishanan, 2014). These reinforcements involved a large proportion of brain in order to make a decision and lead to action, the outcome (Lee et al., 2012).

## 2.6 Mesolimbic dopamine system

This reward pathway is mostly known as mesolimbic pathway (which is also known as mesolimbic dopamine system) (Dreyer, J. L., 2010). This pathway joins VTA to vental striatum located in basal ganglia in the forebrain. The ventral striatum is consists of nucleus accumbens and the olfactory tubercle (Ikemoto, S., 2010).

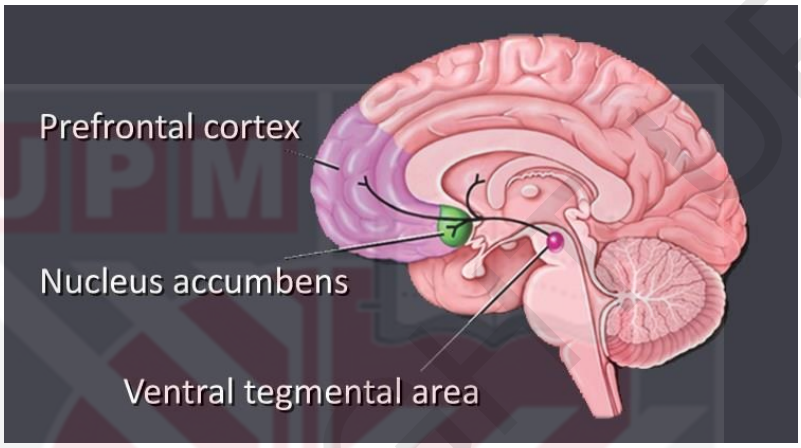


*Figure 6: Rewarding pathway*.

As reward research revolved around dopamine released in the brain, the location where dopamine being released affected the role that it play in this rewarding pathway. In this pathway, dopamine was released in ventral midbrain, particularly at ventral tegmental area (VTA) and later being transmitted to striatal complex. In this case which is a rewarding pathway; dopamine will be projected from ventral tegmental area (VTA) to ventral striatum (Ikemoto, S., 2010).

## 2.7 Dopamine (DA)

Dopamine is a type of neurotransmitter which it is usually released when an individual in a pleasure state. In reward pathway, mesolimbic dopamine system is the one who plays an important role in producing dopamine. Dopamine being produced and released from ventral tegmental area (VTA), particularly in the nerve cells bodies. Dopamine is released to nucleus accumbens and prefrontal cortex. Then, substantia nigra cell bodies take the responsibilities to produce and released dopamine into striatum (Juárez Olguín et al., 2016). This system is called as mesolimbic dopamine system.

Many studies have found that dopamine (DA) is the key player in motivational and this rewarding pathway. It has been associated with motor function in which this neurotransmitter enhances the motivation to act before proceed with the action. Apart from motivation and motor function, it has also been associated with addiction. Dopamine signalling in striatum has always been a parameter either in rewarding, learning and addiction. Mesolimbic dopamine system is served as centre of brain reward pathways in which nucleus accumbens and dopamine transmission are the main component in this rewarding pathway (Martinez et al., 2011). Another pathway of dopamine which are mesostriatal and mesocortical has been found to be associated in reward and addiction, though the dopamine being projected and modulated is differ among those pathways in terms of effect and function changes in addiction (Volkow et al., 2011).

# CHAPTER 3

# MATERIALS AND METHOD

## 3.1 Subjects

Prior to experiment, total of 80 male Sprague Dawley rats which weighed around 250-300g were placed in cages. Each cages housed four rats. Rats were being acclimatised to the laboratory conditions for one week before starting the experiment. Rats were kept in in a room which temperature was regulated and artificial lighting was provided. Food and water were available ad libitum and each animal were handled daily throughout the first week. Experimental testing was then begun seven days after acclimatisation period of the rats and was performed during light cycle. All experiments procedure were conducted at Faculty of Medicine and Health Sciences, particularly at the animal house.

## 3.2 Grouping of rats

Total of 80 rats were divided into two big groups which are for sub-acute study and chronic study. In each of both big groups, it consists of five subgroups in which group one and group two are for positive (morphine) and negative (Tween 80, as vehicle) control, meanwhile group three, four and five are for treatment groups which differentiated by their concentrations, low concentration group (5mg/kg), medium concentration group (20mg/kg) and high concentration group (40mg/kg). For sub-acute study, the period of the study was conducted up to seven days, meanwhile for chronic study; it was conducted up to twenty one days.

## 3.3 Extraction of *Mitragyna speciosa*

### 3.3.1 Preparation of *Mitragyna speciosa*

Raw fresh and mature leaves of *Mitragyna speciosa* was collected from Perlis. Mitragynine that used in the study was extracted from raw *Mitragyna speciosa.* The extraction of mitragynine from leaves of *Mitragyna speciosa* was conducted according to the methods by Houghton and Ikram (1986) with some modification. Firstly, leaves of *Mitragyna speciosa* (1kg) were dried at room temperature below 30 degree Celsius and then it was grinded into dry and powdered form. The powder then was soaked in methanol at room temperature for four days. The mixture then being filtered and methanol was removed by rotary evaporator to give the crude methanolic extract. These procedures were repeated for three times.

### 3.3.2 Preparation of crude alkaloid extract of *Mitragyna speciosa*

An aliquot of the methanol extract dissolved in 10% sulphuric acid, well shaken and left aside for 24 hours. It then was filtered to give the acidic filtrate, in which after that it was being stir with sodium carbonate, made alkaline (pH 9) and lastly mixed with chloroform solvent to extract the alkaloid. The combined chloroform extract will wash with distilled water, dried over anhydrous sodium sulphate and evaporated under reduced pressure at 40 degree Celsius in order to give crude alkaloid extract.

## 3.4 Administration of *Mitragyna speciosa* extract

### 3.4.1 Positive and negative control

Three sets of rats, each set consists of two, were administered with morphine (2mg/kg) dissolved in saline and saline (0.9%) through oral respectively.

### 3.4.2 Sub-acute study

*Mitragyna speciosa* extracts (5, 20 and 40 mg/kg) or vehicles (Tween 80) were administered orally for 7 days before the rats being euthanized. The extract was dissolved in vehicle meanwhile the controls only received the vehicles.

### 3.4.2 Chronic study

*Mitragyna speciosa* extracts (5, 20 and 40 mg/kg) or vehicles (Tween 80) were administered orally for 21 days before the rats being euthanized. The extract was dissolved in vehicle meanwhile the controls only received the vehicles.

## 3.5 Tissue preparation

In order to retrieve the tissues, rats were euthanized and brain was retrieved. The brain was then dissected on ice to obtain the striatum and prefrontal cortex. Striatum and prefrontal cortex were wighed and storedat -80 degree Celsius before the analysis of dopamine (DA), 3, 4-dihydroxyphenylacetic acid (DOPOC) and homovanilic acid (HVA).

This procedure was conducted with deproteinisation in perchloric acid as the sole extraction or purification step before sample was applied to the HPLC system. The tissues then were homogenised and sonicated (Soniprobe 150 at amplitude of 20 microns for 10 seconds) in 500ul ice-cold 0.2M perchloric acid (PCA) containing 0.1% w/v sodium metabisulfite (SMBS), 0.1% w/v cysteine in eppendorfs. Lastly, it was centrifuged (Sanyo Harrier 18/80 at 14,000 rpm) for 20 minutes at 4 degree Celsius. The supernatants were removed and stored for further analysis.

## 3.6 HPLC

All the neurotransmitters were all separated by HPLC and quantified using electrochemical detection. The separation was achieved with the system consisted at a Phenomenox Sphereclone (4.6 x150mm x 5uM) column with Phenomenex C18 4mm security Guard column and an HPLC technology RR/066L solvent pump. The mobile phase consisted of 0.05 M monopotassium phosphate, 0.1 mm EDTA 0.16mM OSS, 16% methanol, 0.05M sodium dihydrogen orthophosphate, 10% acetonitrile and pH 3.0 with phosphoric acid using a flow rate of 0.6ml/min

The mobile phase for HPLC was degas in an ultrasonic bath for 5 minutes before running any samples, to prevent air from entering the columns and detector cells. The mobile phase flow rate was set to 1.0ml/min. The detector system was an Antec Conroller CU-0.4AZ with a glassy carbon working electrode was set at 0.72V vs a Ag/AgCI reference electrode. The sensitivity of the detector was maintained from 0.2 to 1.0nA full scale, depending on the concentration. Then, the sample were loaded onto the HPLC system by injection using a 1ml syringe into the Rhedyne manual injection value fitted with a 20ml sample loop. All neurotransmitter eluted within 30 minutes, I the following oreder DOPAC (6.25mins), dopamine (7.24 mins), HVA (13.59 mins), HVA (15.06 mins) and HVA (20.06 mins).

## 3.7 Neurochemical analysis

The amine values in the samples were calculated by direct comparison between the chromatography peak heights of the sample with the respective standard curve. The DA turnover was calculated as the ratio of DOPAC to DA and DOPAC+HVA to DA. The regional monoamines and metabolites level were expressed as nanogram per gram tissue (ng/g). One- way ANOVA was performed with SPSS followed by a post-hoc Tukey's Multiple Comparison Test where applicable for inter- group comparison. A statistical difference was set at a probability level of $P< 0.05$ for all cases.

# CHAPTER 4

# RESULTS

## 4.1 Sub-acute study

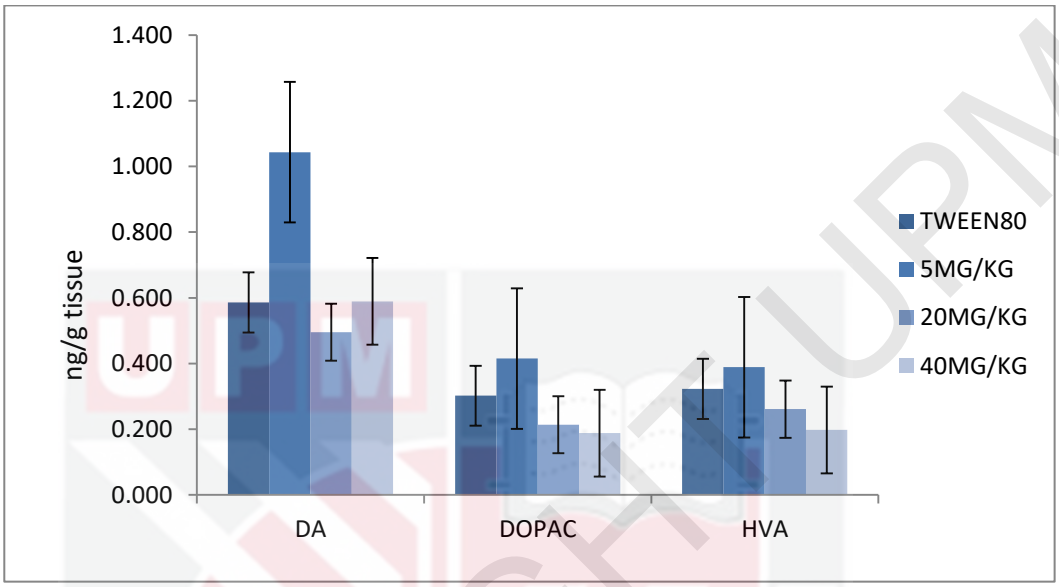


*Figure 7: Regional levels of dopamine and its metabolites (DOPAC and HVA) in the striatum after acute administration of alkaloid extract of Mitragyna Speciosa (5, 20 and 40 mg/kg) compared with vehicle (Tween 80) treated rats.*

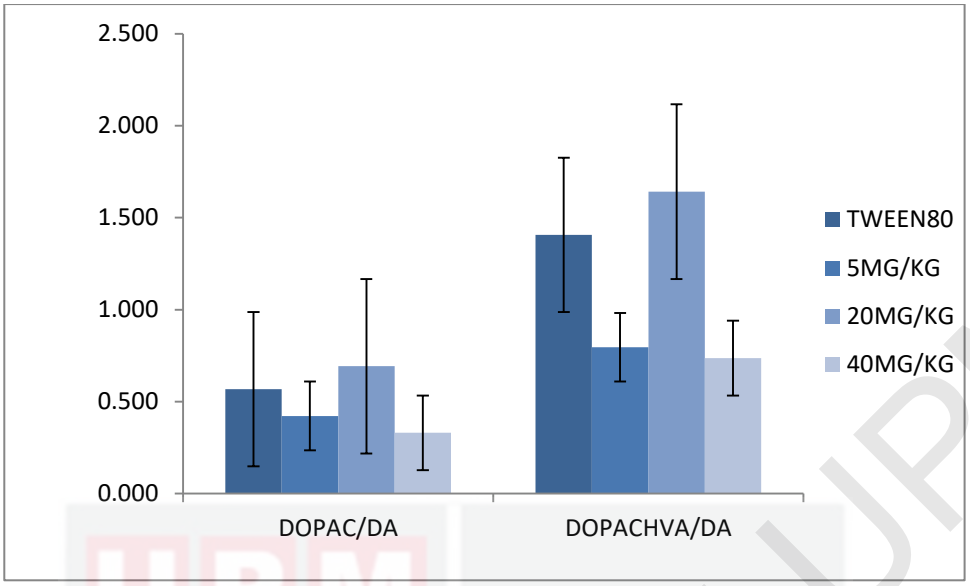


*Figure 8: The ratio of DOPAC and DOPAC+HVA to DA levels in the striatum of rats acutely treated with alkaloid extract of Mitragyna Speciosa (5, 20 and 40 mg/kg) compared with vehicle (Tween 80) treated rats.*

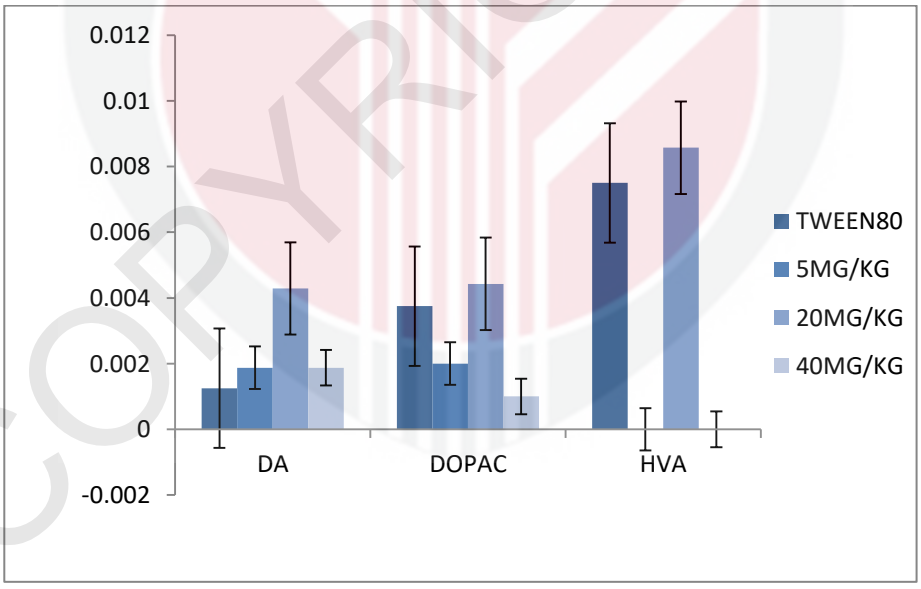


*Figure 9: Regional levels of dopamine and its metabolites (DOPAC and HVA) in the prefrontal cortex after acute administration of alkaloid extract of Mitragyna Speciosa (5, 20 and 40 mg/kg) compared with vehicle (Tween 80) treated rats.*

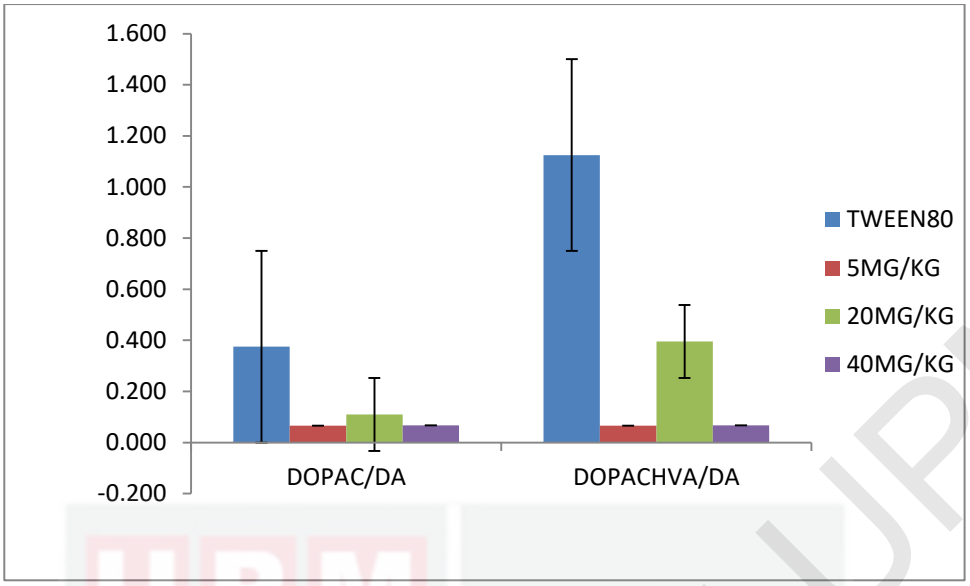


*Figure 10: The ratio of DOPAC and DOPAC+HVA to DA levels in the prefrontal cortex of rats acutely treated with alkaloid extract of Mitragyna Speciosa (5, 20 and 40 mg/kg) compared with vehicle (Tween 80) treated rats.*

Figure 7 and 9 show the regional levels of dopamine and its metabolites which are DOPAC and HVA in the striatum and prefrontal cortex after acute administration of alkaloid extract of *Mitragyna speciosa* with concentrations of 5, 20 and 40mg/kg compared with vehicle administered (Tween 80) treated rats. The extract was administered in a period of 7 days before the rats being euthanized. Figure 8 and 10 show the ratio of DOPAC and DOPAC+HVA to DA levels in striatum and prefrontal cortex of rats after acute administration of alkaloid extract of *Mitragyna speciosa* with concentration of 5, 20 and 40mg/kg compared with vehicle (Tween 80) treated rats.

## 4.2 Chronic study

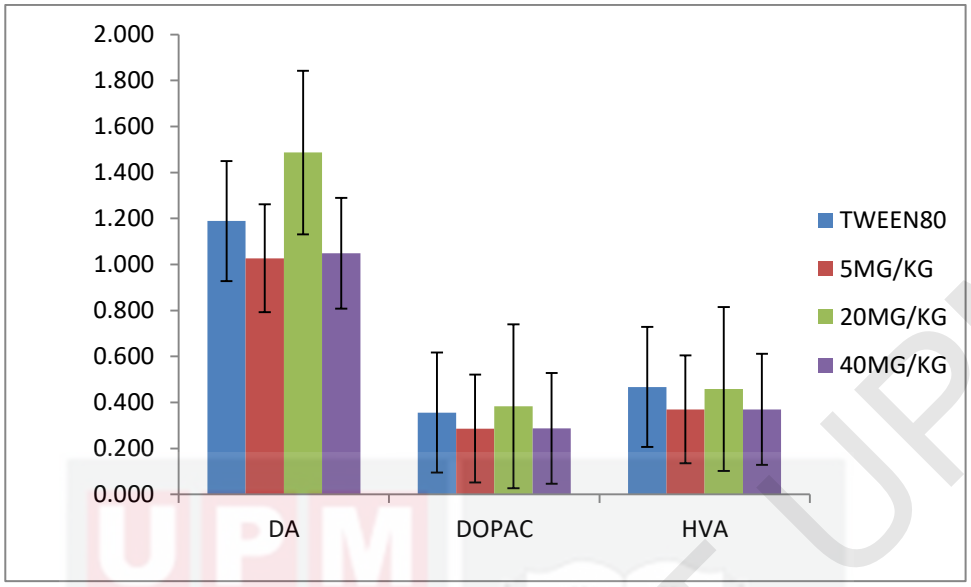


*Figure 11: Regional levels of dopamine and its metabolites (DOPAC and HVA) in the striatum after chronic administration of alkaloid extract of Mitragyna Speciosa (5, 20 and 40 mg/kg) compared with vehicle (Tween 80) treated rats.*

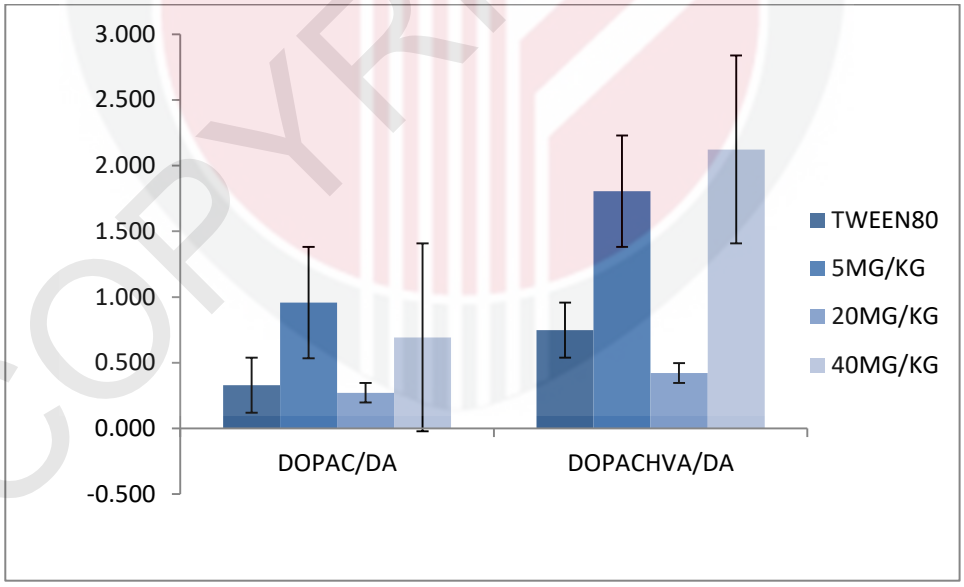


*Figure 12: The ratio of DOPAC and DOPAC+HVA to DA levels in the striatum of rats chronically treated with alkaloid extract of Mitragyna Speciosa (5, 20 and 40 mg/kg) compared with vehicle (Tween 80) treated rats.*

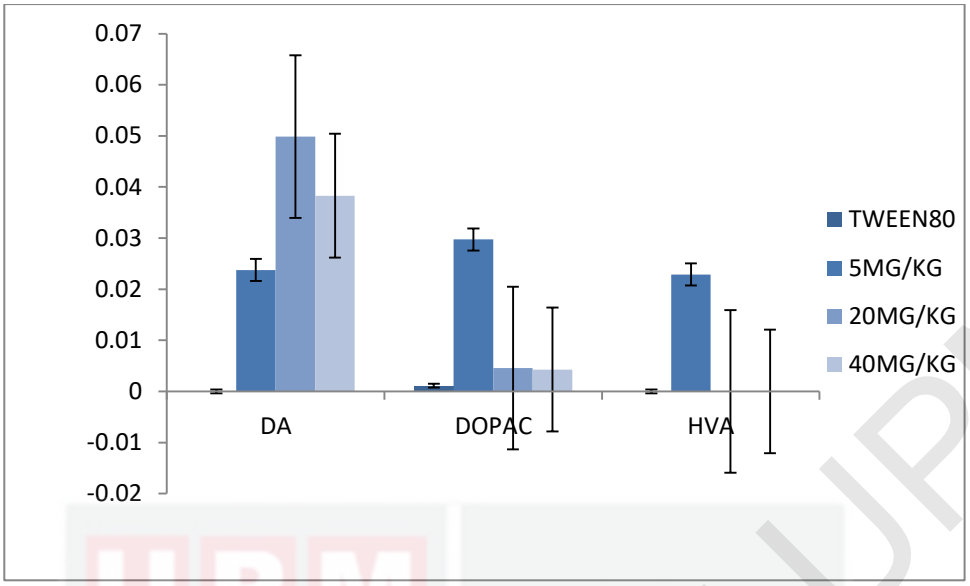


*Figure 13: Regional levels of dopamine and its metabolites (DOPAC and HVA) in the prefrontal cortex after chronic administration of alkaloid extract of Mitragyna Speciosa (5, 20 and 40 mg/kg) compared with vehicle (Tween 80) treated rats.*

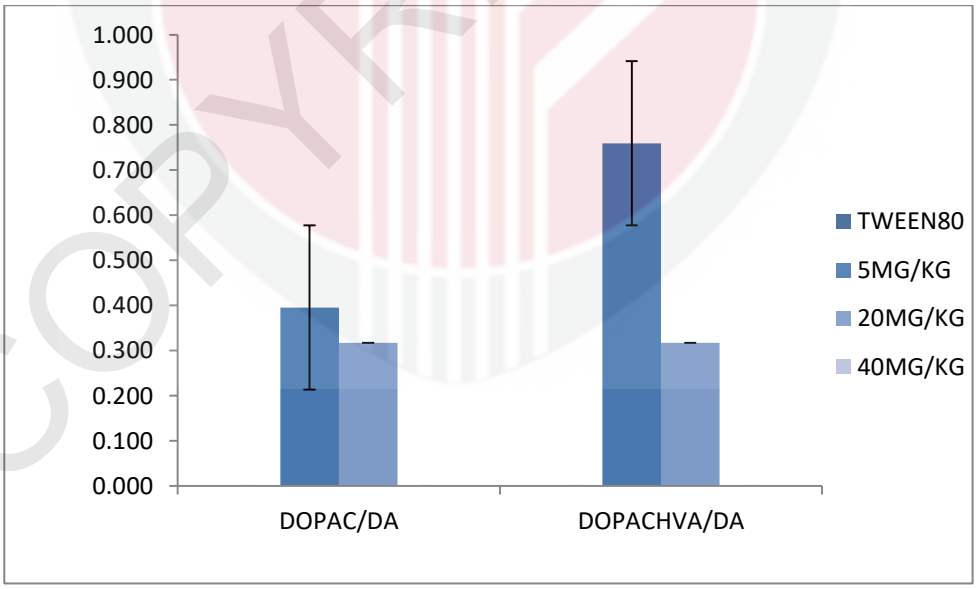


*Figure 14: The ratio of DOPAC and DOPAC+HVA to DA levels in the prefrontal cortex of rats chronically treated with alkaloid extract of Mitragyna Speciosa (5, 20 and 40 mg/kg) compared with vehicle (Tween 80) treated rats.*

Figure 11 and 13 show the regional levels of dopamine and its metabolites which are DOPAC and HVA in the striatum and prefrontal cortex after chronic administration of alkaloid extract of *Mitragyna speciosa* with concentration of 5, 20, and 40mg/kg compared with vehicle (Tween 80) treated rats. The extract was administered in a period of 21 days before the rats being euthanized. Figure 12 and 14 show the ratio of DOPAC and DOPAC+HVA to DA levels in the striatum and prefrontal cortex of rats after chronic administration of alkaloid extract of *Mitragyna speciosa* with concentration of 5, 20 and 40mg/kg compared with vehicle (Tween 80) treated rats.

# CHAPTER 5

# DISCUSSION

In the present study, the dopamine and its metabolite which are DOPAC and HVA level was measured after administering the *Mitragyna speciosa* extract in the brain of Sprague Dawley rat particularly in striatum and prefrontal cortex region. From the results, levels of dopamine and its metabolite in striatum and prefrontal cortex shows an increment at low and medium concentration of *Mitragyna speciosa* methanol extracts for acute administration. Looking at the chronic administration results, distinct increment of dopamine and its metabolite level can be seen in both striatum and prefrontal cortex. However, there is no increment for high doses or concentration. According to Boyer et al, 2008, mitragynine significantly inhibits the receptor for dopamine in radioligand binding assay. This would lead to the reduced number of dopamine receptor, thus, suggesting elevated dopamine released in the brain (Diana M., 2011). Increase in dopamine in brain's ventral striatum gives out the sensation of reward (Volkow et al., 2010). This activity is known as antipsychotics, which is also known as neuroleptics.

In this present study, it can be seen that dopamine is being released or fired in short burst firing. According to Volkow et al, 2010, dopamine signalling exists in two forms which are phasic and tonic. Phasic form is characterised by high amplitude and short burst firing, meanwhile, tonic form features low amplitude and sustained period of time. Hence, in this study, the dopamine firing can be categorised into phasic form of firing as it is in short period of time and in high amplitude. This phasic form of dopamine signalling is required

characteristic in drug abuse in order to stimulate the conditioned responses (Volkow et al., 2010).

# CHAPTER 6

# CONCLUSION

## 6.1 Conclusion

In conclusion, there is an increase in dopamine and its metabolite level in rewarding pathway as the receptors for dopamine is being blocked due to mitragynine. *Mitragyna speciosa* have shown antipsychotic activity in which it may be used for slowing down of withdrawal symptom of drugs.

## 6.2 Recommendation

Further studies are needed to determine on how *Mitragyna speciosa* affect the brain particularly on behavioural study of an animal to observe a significant effect of administrating *Mitragyna speciosa*.

## 6.3 Limitation

Due to time restriction caused by pandemic, I was unable to conduct the research project by my own. I only received several graphs of analysed raw data. Hence, I was unable to run statistical analysis by myself.

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