

**MAGNETIC RESONANCE IMAGING
BRAIN VOLUMETRY AMONG
ADULT MALAY MALE
REGULAR KRATOM LEAVES USERS.**

DR. SITI DAYANA BINTI MOHAMAD

**DISSERTATION SUBMITTED IN PARTIAL
FULFILMENT OF THE REQUIREMENT FOR
MASTER OF MEDICINE (RADIOLOGY)**



UNIVERSITI SAINS MALAYSIA

2022

ACKNOWLEDGEMENT

I would like to express my gratitude to Allah SWT for giving me the opportunity and endlessly guiding me throughout the completion of this thesis. The completion of this undertaking could also not have been possible without the participation, cooperation, and assistance of so many parties whose names may not all be enumerated. Their contributions are sincerely appreciated and gratefully acknowledged. However, I would like to express deep and utmost appreciation, particularly to the following:

1. Dr Muhamad Zabidi bin Ahmad as my supervisor, who gave assistance and provided guidance in carrying out this study until its completion.
2. Dr Darshan Singh Mahinder Singh from Centre for Drug Research who have played an important role in making this study possible in the first place.
3. Prof Dr Mohd Shafie Abdullah, Prof Madya Dr Mohd Ezane Aziz, Prof Madya Dr Juhara Haron, Dr Khairil Amir Sayuti, Dr Chandran Nadarajan, Dr Ahmad Hadif Zaidin Samsudin, Dr Wan Aireene Wan Ahmed, Dr Nur Asma Sapiai, and Dr Nasibah Mohamad (Lecturers cum radiologists), all of whom directly or indirectly contributed their ideas and comments to the success of this study.
4. Radiographers, colleagues, and all the staff in the Department of Radiology in Hospital USM, Kubang Kerian, Kelantan and Radiology Department, Advance Medical and Dental Institute (IPPT Bertam), Penang.
5. My dearest husband, Dr Muhamad Fakhurrazi bin Mohamad Bakri, my mother, Puan Zarimah binti Zakaria, my family, colleagues, and my dearest friends who have shown tremendous support and understanding throughout this journey.

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT	i
TABLE OF CONTENTS	ii
LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS	iv
LIST OF FIGURES	v
ABSTRAK	vi
ABSTRACT	viii
CHAPTER 1: BACKGROUND	1
1.1 Introduction	1
1.2 Objective	5
1.2.1 General objective:	5
1.2.2 Specific objectives:	5
1.3 Hypothesis	5
1.4 Research Question	6
CHAPTER 2: LITERATURE REVIEW	7
2.1 Brain	7
2.2 Brain Volumetry	11
2.3 Kratom	13
2.4 Kratom studies in humans	16
2.5 Potential effects of Kratom on the brain	17
2.6 Conceptual Framework	20
2.7 Study Rationale	21

CHAPTER 3: METHODOLOGY	22
3.1 Research Design	22
3.2 Study area and study population:	22
3.3 Subject criteria	23
3.3.1 Inclusion criteria:	23
3.3.2 Exclusion criteria:	23
3.4 Sampling method	23
3.5 Sample size calculation	24
3.6 Research Tools:	28
3.7 Operational Definition	28
3.8 Data collection	29
3.8.1 Patient Cohort	29
3.9 Confidentiality and Privacy	45
3.10 Ethical Consideration	45
3.11 Study Flow Chart	47
CHAPTER 4: MANUSCRIPT	48
CHAPTER 5: REFERENCES	90
CHAPTER 6: APPENDICES	101
6.1 Appendix A: Data Collection Sheet	101
6.2 Appendix B: Subject Data Sheet	103
6.3 Appendix C: Human Ethical Approval	107
6.4 Journal format	109
6.5 Raw Data (CD)	116

LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

BD	Bipolar disorder
CNS	Central nervous system
FS	Freesurfer
ICV	Intracranial volume
ml	millilitre
MRI	Magnetic resonance imaging
PACS	Picture archiving and communication system
SD	Standard deviation
SPM12	Statistical Parametric Mapping 12
TIV	Total intracranial volume
TBV	Total brain volume
US	United States

LIST OF FIGURES

	Page
Figure 1	Anterior view of the skull 8
Figure 2	Lateral view of the skull 9
Figure 3	Cranial meninges 10
Figure 4	Dural partitions. A. Diagram. B. Dissection. 11
Figure 5	A. Kratom leaves. B. Mitragynine (major indole alkaloid of Kratom). C. The other indole alkaloids of Kratom. 15
Figure 6	Conceptual framework 20
Figure 7	MR image showing landmarks for measurement of ICV 33
Figure 8	Example of manual tracing delineation of intracranial area. 35
Figure 9	Example of manual tracing delineation of brain surface area. 36-38
Figure 10	Example of manual tracing of caudate nucleus and putamen. 40-42
Figure 11	Study flow chart 47

ABSTRAK

Latar belakang: *Mitragyna speciosa* (Korth.) atau lebih dikenali sebagai kratom adalah tumbuhan asli yang biasa ditemui di Asia Tenggara dengan sejarah panjang penggunaan tradisional sebagai ubat herba untuk pelbagai penyakit, biasanya digunakan sebagai ubat penahan sakit dan untuk memerangi keletihan. Terkini, selain daripada manfaat perubatan, ia telah mendapat populariti untuk digunakan sebagai ubat rekreasi dan sebagai pengganti opioid. Walaupun penggunaan semakin meningkat di seluruh dunia, potensi kesan sampingan penggunaan kratom yang berpanjangan pada morfometri otak, pada masa ini masih tidak jelas.

Metod: Dua puluh dua orang pengguna kratom dewasa dan tiga puluh orang bukan pengguna kratom (kumpulan kawalan) yang menjalani pengimejan resonans magnetik otak (MRI) untuk penyelidikan terdahulu telah dimasukkan dalam kajian keratan rentas ini. Pengesanan manual isipadu otak untuk jumlah isipadu intrakranial (TIV atau ICV), jumlah isipadu otak (TBV), isipadu nukleus caudate dan isipadu putamen telah dilakukan untuk semua peserta. Perbezaan dalam nilai purata untuk isipadu intrakranial (TIV atau ICV), isipadu otak (TBV), isipadu nukleus caudate dan isipadu putamen antara pengguna kratom dan bukan kratom ditentukan menggunakan ujian-t bebas statistik.

Keputusan: Purata bagi jumlah isipadu intrakranial (SD) dan isipadu otak (SD) dalam kalangan pengguna kratom dewasa masing-masing ialah 1502.28 (95.41) cm³ dan 1388.63 (118.03) cm³. Bagi subjek pengguna kratom, purata (SD) bagi isipadu nukleus caudate kanan, kiri dan isipadu keseluruhan nukleus caudate yang dilaporkan ialah 3.89 (0.48) cm³, 3.96 (0.49) cm³ dan 7.86 (0.95) cm³. Manakala, bagi purata isipadu putamen

kanan, kiri dan jumlah keseluruhan putamen, purata isipadu (SD) yang dilaporkan masing-masing ialah 4.31 (0.29) cm³, 4.37 (0.26) cm³ dan 8.67 (0.52) cm³. Tiada perbezaan yang ketara ($p > 0.05$) dalam purata jumlah isipadu intrakranial (TIV atau ICV) dan jumlah isipadu otak (TBV) antara pengguna kratom dan kawalan. Walau bagaimanapun, terdapat peningkatan dalam isipadu purata nukleus caudate dan isipadu purata putamen ($p < 0.05$) bagi pengguna kratom berbanding kawalan.

Kesimpulan: Kajian ini menunjukkan penggunaan jangka panjang kratom dikaitkan dengan struktur otak subkortikal (nukleus caudate dan putamen) yang diubah dalam pengguna kratom biasa dalam tetapan tradisional. Walau bagaimanapun, kajian lanjut diperlukan untuk mewujudkan lebih banyak data untuk kegunaan kratom dan kesannya berkaitan dengan morfometri otak.

Kata kunci: *Mitragyna speciose*, *mitragynine*, *ketum*, *pengimejan resonans magnetik*, *otak*, *kajian isipadu*, *opioid*.

ABSTRACT

Backgrounds: *Mitragyna speciosa* (Korth.) or commonly known as kratom, is an indigenous plant commonly found in Southeast Asia with a long history of traditional use as herbal remedies for various ailments, usually consumed as painkillers and to combat fatigue. More recently, apart from the medicinal benefit, it has gained popularity for use as a recreational drug and as an opioid substitute. Despite the increasing usage across the globe, the potential side-effects of prolonged kratom use on brain morphometry, however, are currently unclear.

Methods: Twenty-two regular adult kratom users and thirty non-kratom users who underwent brain magnetic resonance imaging (MRI) for prior research were included in this cross-sectional study. Manual tracing of brain volumetry for total intracranial volume (TIV or ICV), total brain volume (TBV), caudate nucleus, and putamen were performed for all the participants. The difference in means for TIV, TBV, caudate nucleus, and putamen between kratom and non-kratom users were determined using an independent statistical t-test.

Results: The mean (SD) total intracranial volume and mean total brain volume (SD) among regular adult kratom leave users were 1502.28 (95.41) cm³ and 1388.63 (118.03) cm³, respectively. For kratom user subjects, the mean (SD) for the right, left, and total caudate nucleus volumes reported were 3.89 (0.48) cm³, 3.96 (0.49) cm³, and 7.86 (0.95) cm³, respectively. While for the right, left and total putamen volume, the mean (SD) reported were 4.31 (0.29) cm³, 4.37 (0.26) cm³ and 8.67 (0.52) cm³ respectively. There were no significant differences ($p>0.05$) in the mean total intracranial volume (TIV or

ICV) and total brain volume (TBV) between kratom users and the controls. However, there was an increase in mean caudate nucleus and putamen volume ($p < 0.05$) for kratom users relative to the controls.

Conclusion: This study showed long-term consumption of kratom is associated with altered subcortical brain structures (caudate nucleus and putamen) in regular kratom users in traditional settings. However, further study is needed to establish more data on kratom use and its effects with regard to brain morphometry.

Keywords: Mitragyna speciosa, mitragynine, kratom, magnetic resonance imaging, brain, volumetry, opioid.

CHAPTER 1: BACKGROUND

1.1 Introduction

Mitragyna speciosa Korth from the Rubiaceae family or more commonly known as kratom or ‘ketum’ (aka *thang*, *kakuam*, *thom*, *biak biak*), is a plant native to Southeast Asia, the leaves of which have been used for many purposes for many decades. In Southeast Asia, humans have traditionally used it to treat many ailments, and it is typically consumed as tea. A cross-sectional survey among 293 regular Kratom users in 2013 has shown that among the reasons for Kratom usage were to enhance physical energy, curiosity, peer influence, to abstain from illicit drugs/alcohol, self-treatment, to improve mood/ease boredom, and to relieve fatigue (Singh, Müller and Vicknasingam, 2014). This study has reported that more than one-third used Kratom because of curiosity and peer influence. About 15% (45/293) used Kratom to abstain from illicit drugs and alcohol, while about 13% (38/293) used Kratom to treat their medical problems (e.g., diabetes). In short, Mitragynine demonstrates addictive liabilities and impairs cognition while at the same time being able to alleviate the withdrawal symptoms and exhibits potential medical applications (including analgesic properties) (Suhaimi *et al.*, 2016). Kratom has been traditionally used to treat various ailments; however, it has growingly been used for recreational purposes (Singh, Narayanan and Vicknasingam, 2016).

Despite its many benefits, Kratom is well known to cause dependence among its regular users. In a study in 2013, those who consumed more than three glasses per day reported severe dependence compared to those who consumed less than three glasses per day. Regular Kratom users also developed physiological and psychological withdrawal

symptoms during the first day of Kratom cessation. Among physiological withdrawal symptoms encountered by Kratom users during withdrawal include sleeping difficulty, decreased appetite, nausea, vomiting, muscle spasm, sweating, fever, abdominal pain, diarrhoea, headaches, hot flashes, watery eyes and nose, hiccups, and shakiness or tremors and psychological withdrawal symptoms commonly reported include nervousness, sadness, restlessness, anger, tension, and depressed mood (Singh, Müller and Vicknasingam, 2014). It was also reported that high craving coincided with high Kratom consumption but was not dependent on the length of the consumption (Singh, Müller and Vicknasingam, 2014).

Despite being thought that Kratom is more commonly used in Southeast Asia, it seems that Kratom has also gained popularity in other countries, including the United States (US). An anonymous online survey with 2798 respondents that was conducted in the US has reported that Kratom is used among White, middle-aged Americans for symptoms of pain, anxiety, depression, and opioid withdrawal. Routes of administration vary and include capsule/pill, prepared as tea/beverages, ingested powder and other routes (e.g., smoked, intravenous/subcutaneous, consumed as extract), with the most common route of administration being as an ingested powder (43.6 percent).

The molecular constituents of Kratom have been extensively studied. It was reported that there are more than 40 indole alkaloids have been identified within the plant, with the indole alkaloid mitragynine has been universally cited as the major alkaloids constituents of Kratom (E Adkins, W Boyer and R McCurdy, 2011), joined by 7-hydroxymitragynine (7-OH) at a much lower concentration. It was reported that

mitragynine and 7-OH are partial agonists of the human μ -opioid receptor (hMOR) in vitro, with 7-OH being ~10-fold more potent than mitragynine. Both compounds are also known to exhibit opioid- receptor-dependent analgesic effects in rodent models and are G protein-biased agonists of the mu-opioid receptor (MOR). Given these special properties, kratom alkaloids were classified as “atypical opioids”, and it has been postulated that these compounds may provide a better therapeutic window between analgesia and classical opioid side effects (E Adkins, W Boyer and R McCurdy, 2011; Kruegel *et al.*, 2016).

Decades of studies and researches pertaining to Kratom has focused predominantly on exploring the pharmacokinetic properties of Kratom (Rech *et al.*, 2015; Trakulsrichai *et al.*, 2015; Suhaimi *et al.*, 2016; Warner, Kaufman and Grundmann, 2016; Brown, Lund and Murch, 2017; Hamid, Ramli and Yusoff, 2017; Kruegel and Grundmann, 2018; Kamble *et al.*, 2019; Kruegel *et al.*, 2019), its hematological and clinical chemistry (D Singh *et al.*, 2018), socio-demographics of regular Kratom users (Grundmann, 2017; Garcia-Romeu *et al.*, 2020), the varieties of motives of Kratom usage (Trakulsrichai *et al.*, 2013; Cinosi *et al.*, 2015; Singh, Narayanan and Vicknasingam, 2016; Coe *et al.*, 2019), the physiological and psychological effects of Kratom use/abuse (Hassan *et al.*, 2013; Trakulsrichai *et al.*, 2013; Singh, Müller and Vicknasingam, 2014; Stolt *et al.*, 2014; Singh *et al.*, 2015; Swogger *et al.*, 2015; Yusoff *et al.*, 2016; Swogger and Walsh, 2018; Hemby *et al.*, 2019; Post *et al.*, 2019) and legislative policies regarding Kratom (Henningfield, Fant and Wang, 2018; Prozialeck *et al.*, 2019). In addition, case reports mainly of adverse effects of Kratom usage have also been published, especially in the West and less commonly in Southeast Asia (Neerman, Frost and Deking, 2013; McIntyre *et al.*, 2015; Singh, Narayanan and Vicknasingam, 2016).

It has been established that substance abuse or long term usage of certain drugs (for example, alcohol, cocaine and opioids) may cause structural changes to the human brain. A study involving individuals with chronic low back pain who received a daily dose of morphine for one month revealed reduced grey matter volume in the amygdala (Younger *et al.*, 2011). Another study in 2010 also reported atrophy in the amygdala among opioid-dependent individuals (Upadhyay *et al.*, 2010). Despite the increasing use of Kratom in the world, unfortunately, up to this date, there are very limited studies that evaluate the structural changes of the brain following long term or regular Kratom usage.

This study aims to determine if there is a significant difference in brain volumetry among regular adult Kratom users compared to their non-Kratom user counterparts. The focus would be on evaluating the total intracranial volume, brain volume, and subcortical brain volume (focusing on the caudate nucleus and putamen). By assessing these brain volumetry values, we hope this would be an initial step in determining the effects of regular Kratom usage on brain morphology.

1.2 Objective

1.2.1 General objective:

To investigate the brain volumetry among regular adult Kratom leaves users.

1.2.2 Specific objectives:

1. To determine the mean brain volumes (total intracranial volume, total brain volume, volumes of caudate nucleus, putamen) among regular adult Kratom leaves users.
2. To compare the mean total intracranial volume and total brain volume between regular Kratom users and non-Kratom users.
3. To compare the mean volume of caudate nucleus and putamen between regular Kratom users and non-Kratom users.

1.3 Hypothesis

1. There is significant difference in brain volumetry between regular Kratom leaves users and non-Kratom leaves users.
2. There is significant difference in the mean total intracranial volume and total brain volume between regular Kratom users and non-Kratom users.
3. There is significant difference in the mean volume of caudate nucleus and putamen between regular Kratom users and non-Kratom users.

1.4 Research Question

1. Does regular Kratom usage cause any significant alterations in the brain volumes?
2. Does regular Kratom usage cause any significant alteration in the mean total intracranial volume and total brain volume?
3. Does regular Kratom usage cause any significant alteration in the mean volume of caudate nucleus and putamen?

CHAPTER 2: LITERATURE REVIEW

2.1 Brain

The cranial cavity is the largest compartment in the head and contains the brain. The brain is one of the most complex structures in the human body and is confined within the skull. There are 22 bones in the skull itself. Except for the mandible, the bones in the skull are immobile and attached by sutures to form the cranium. The calvarium comprises of bones, namely paired temporal and parietal bones, parts of the unpaired frontal, sphenoid, and occipital bones (Figure 1 and Figure 2). The bones that formed the cranium base are mainly parts of sphenoid, temporal, and occipital bones (Drake, Vogl and Mitchell, 2015).

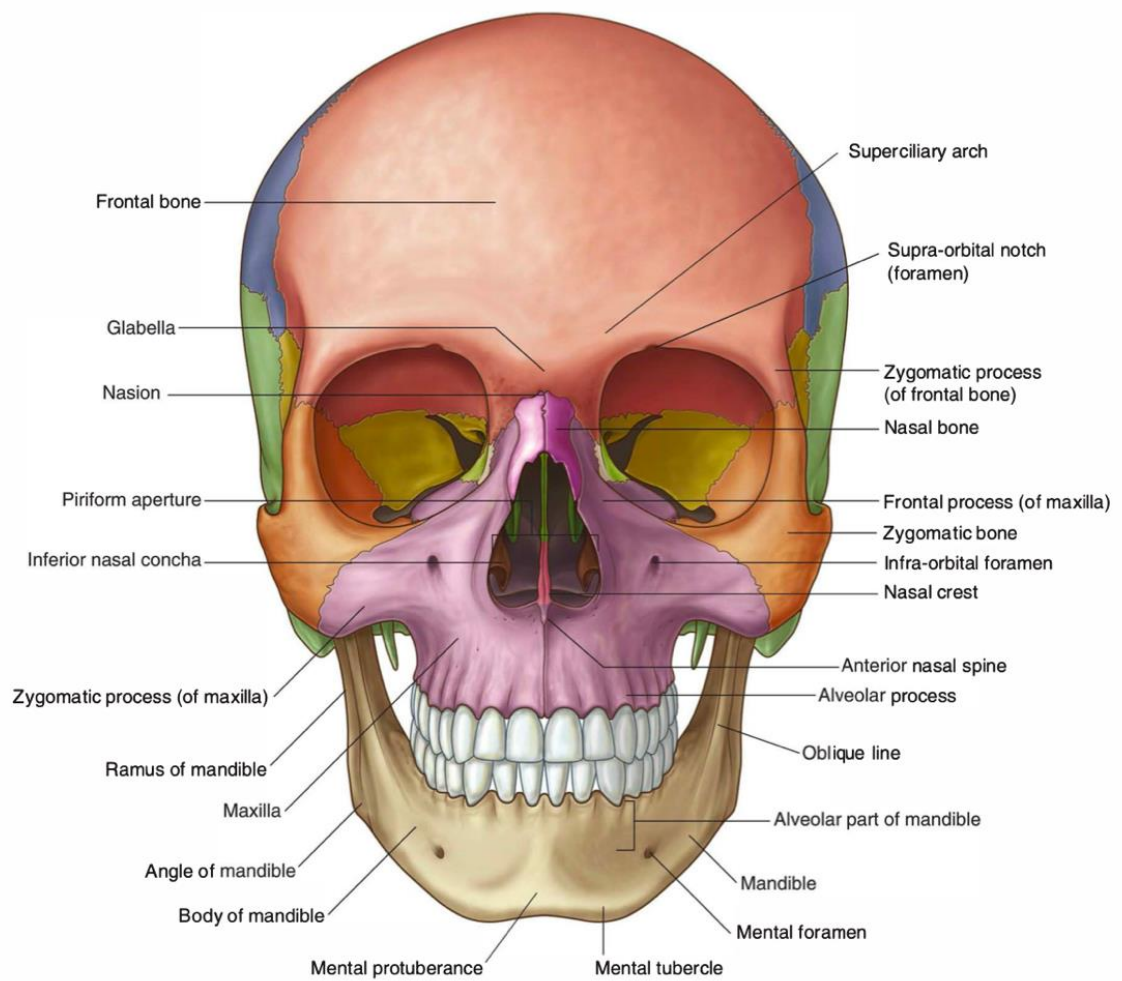


Figure 1: Anterior view of the skull (Adapted from Drake, Vogl and Mitchell, 2015)

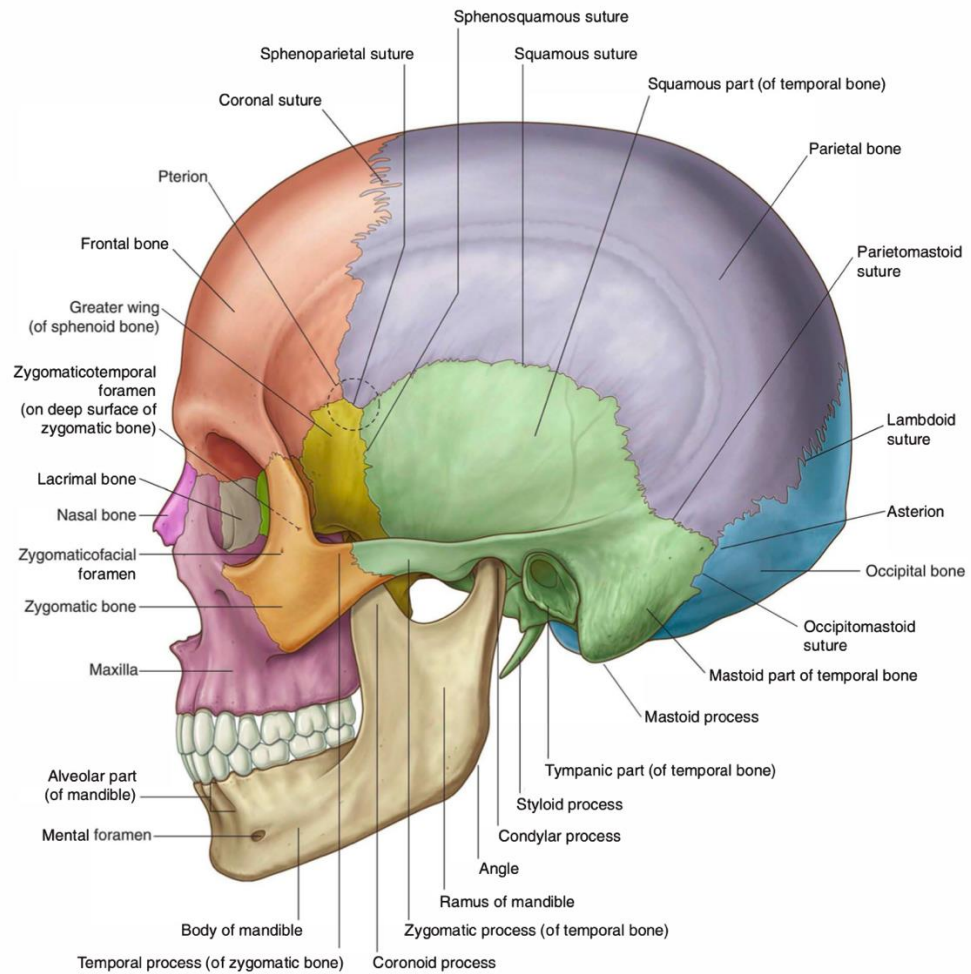


Figure 2: Lateral view of the skull. (Adapted from Drake, Vogl and Mitchell, 2015)

There are three layers of membrane (meninges) that surround the brain, namely the dura mater (a tough, outer layer), the arachnoid mater (delicate middle layer) and an inner layer called pia mater, which firmly attaches to the brain surface (Figura 3) (Drake, Vogl and Mitchell, 2015).

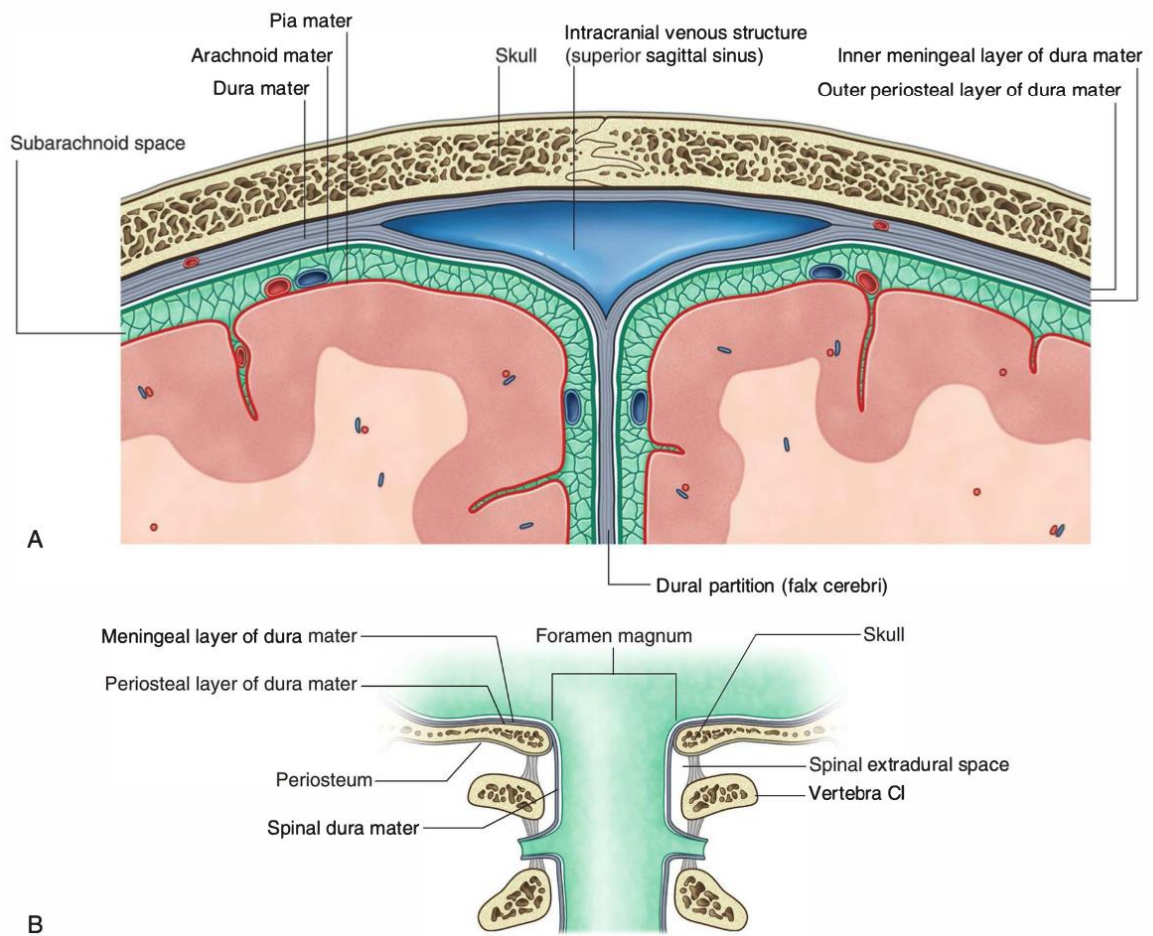


Figure 3: Cranial meninges. (Adapted from Drake, Vogl and Mitchell, 2015)

Apart from the different bones forming the skull cavity, within the cranial cavity, there are also dural partitions that project into the cranial cavity, partially subdividing the cranial cavity. These include the falx cerebri, tentorium cerebelli, falx cerebelli, and diaphragma sellae (Figure 4) (Drake, Vogl and Mitchell, 2015).

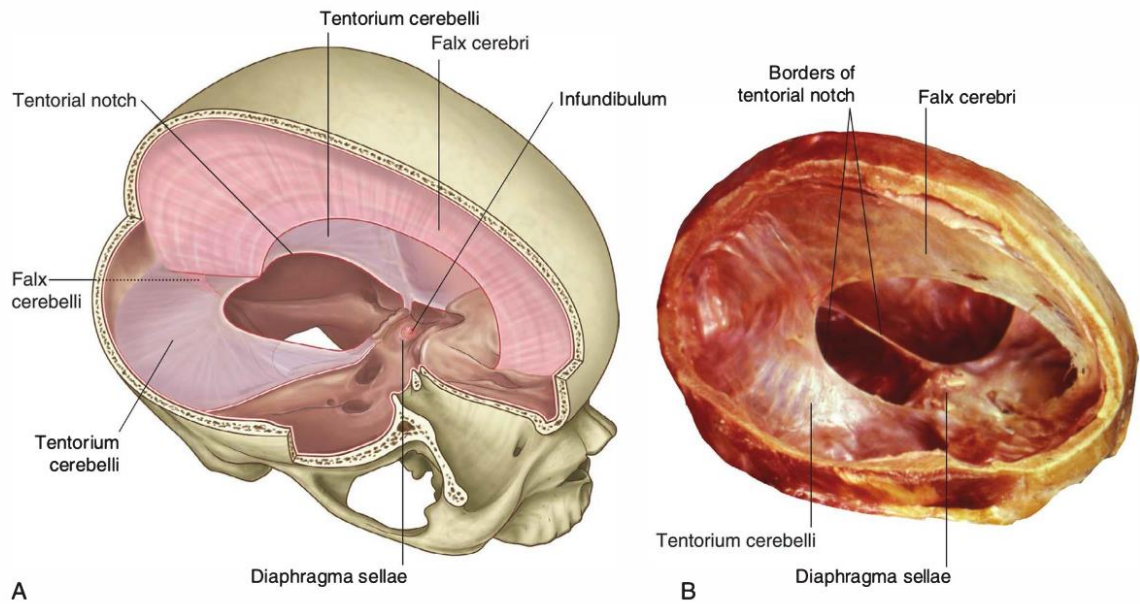


Figure 4: Dural partitions. A. Diagram. B. Dissection. (Adapted from Drake, Vogl and Mitchell, 2015)

2.2 Brain Volumetry

The advances in current technology especially in magnetic resonance imaging (MRI) have allowed us to study the brain in-vivo and not just limited to post-mortem subjects. In a structural based evaluation of the brain on MRI studies, profound changes can be assessed, including atrophy, lacunes, white matter lesion, dilated perivascular spaces, microinfarcts and microbleeds. The pathophysiology of brain atrophy encompasses loss of myelinated nerve fibres, reduction of dendrites and synapses, or death of neurons. Brain atrophy can occur in many conditions, such as in normal ageing, various neurodegenerative conditions, prior brain trauma and consumption of certain drugs or toxins (for example, in cases of alcohol abuse) (Grajauskas *et al.*, 2019).

There are numerous studies available up to this date that explores brain volumetry and brain segmentation in different pathology or conditions (Bagci *et al.*, 2013; Noyan *et*

al., 2016; Grajauskas *et al.*, 2019; Sander *et al.*, 2019; Billot *et al.*, 2020). A study (Germeyan *et al.*, 2014) compares automated and manual hippocampal segmentation in preoperative and postoperative patients with epilepsy. A study in 2016 by (Hibar *et al.*, 2016) investigated ICV and eight subcortical brain measurements in bipolar disorder (BD) patients and found that there are volumetric reductions in BD patients for mean hippocampus and thalamus with enlarged lateral when compared to the controls group. At the same time, a study in 2013 by (Gordillo, Montseny and Sobrevilla, 2013) explored brain segmentation techniques in brain tumour cases.

Volumetry measurements can be utilised to evaluate structural changes in brain volumes. There are many methods available for brain volumetry measurements which include manual, semi-automated and fully automated methods. Manual brain segmentation remains the gold standard of brain volumetry (Karaca *et al.*, 2020); however, it is time-consuming and labour intensive to be carried out.

In brain volumetric studies and brain segmentation, among the two most common fully automated methods are Statistical Parametric Mapping (SPM, version 8, (Friston and Ashburner, 2007)) and Freesurfer (FS, version 5.1.0, (Dale, Fischl and Sereno, 1999)). Fully automated methods of brain segmentation are not as labour intensive and time-consuming as manual methods; however, they may not be as accurate as their manual counterparts. A study by (Nordenskjöld *et al.*, 2013) involving 399 elderly subjects comparing intracranial volume estimations between SPM and FS shows that both of these methods overestimated the ICV. The study also revealed that SPM showed bias associated with gender and atrophy while FS showed bias dependent on skull size (Nordenskjöld *et al.*, 2013). There was a study in 2015 involving 288 subjects in a multi-

centre clinical trial in Alzheimer's disease, which evaluated Statistical Parametric Mapping 12 (SPM12) automated segmentation for TIV measurement in place of manual segmentation and also compared it with SPM8 and FreeSurfer 5.3.0. (Malone *et al.*, 2015). It was established that there is a high correlation between SPM12 TIV and manual TIV, which was significantly higher for either SPM8 or FreeSurfer, suggesting that SPM12 TIV estimates are an acceptable alternative for labour-intensive manual brain estimates.

2.3 Kratom

Kratom (Figure 5) or its scientific name *Mitragyna speciosa* Korth. is a tropical medicinal plant that is indigenous to Southeast Asia (D Singh *et al.*, 2018). Kratom or ketum is the most common term that refers to the leaves (whole, chopped, or powdered) and leaf extracts (e.g., tea-like brews and commercially prepared liquids) of the *Mitragyna speciosa* tree (Henningfield, Fant and Wang, 2018). It has been traditionally used for decades in Southeast Asia, especially in Malaysia, Thailand and the Philippines for its many perceived benefits (D Singh *et al.*, 2018).

Kratom is a part of the Rubiaceae family, which also includes the genus *Coffea* (Eisenman, 2014). Like the coffee tree, kratom contains numerous alkaloids, some of which are active in the central nervous system (CNS) and can produce diverse physiological and behavioural effects (Henningfield, Fant and Wang, 2018). About 44 alkaloids have been isolated from kratom leaves, and the indole alkaloid mitragynine was found to be the major alkaloid constituent of Kratom (Yusoff *et al.*, 2016). A minor constituent 7- hydroxymitragynine has been studied and reported to have unique opioid

like-effects (Yusoff *et al.*, 2016). The other major alkaloids of Kratom (Figure 5) are paynantheine, speciogynine, and speciociliatine with significant variation in the quantities of these four major alkaloids depending on the different regional varieties of the plant, and are also dependent on plant age (E Adkins, W Boyer and R McCurdy, 2011; Kruegel and Grundmann, 2018). These findings further complicate the interpretation of reported psychoactive and medicinal effects from the raw plant material. The mitragynine compound is found to bind mu- and kappa-opioid receptors but has additional receptor affinities that might alter its effectiveness at mitigating opioid withdrawal (Boyer *et al.*, 2008). Unlike opioid counterparts, Kratom and its alkaloids do not present with significant respiratory depression (Kruegel and Grundmann, 2018).

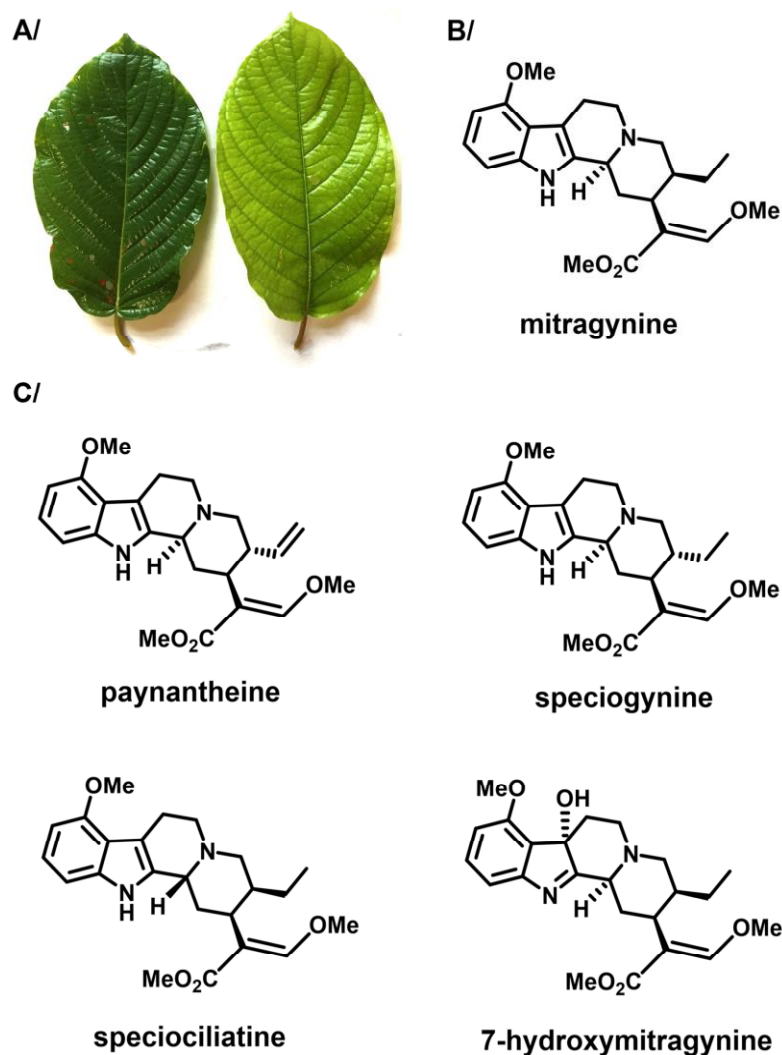


Figure 5: A. Kratom leaves. B. Mitragynine (major indole alkaloid of Kratom). C. The other indole alkaloids of Kratom. (Adapted from Kruegel and Grundmann, 2018).

Rural populations have used kratom in Southeast Asia for both cultural and recreational properties. It is readily available in local coffee shops in Southeast Asia compared to the online market in the West, users in both regions have shifted to recreational purposes (Singh, Narayanan and Vicknasingam, 2016). Among its many functions is an opioid substitute, stimulating or mood-enhancing effects, pain relief, and a folk remedy for various ailments (Singh *et al.*, 2019). Kratom was also shown to

produce dose-dependent psychotropic effects, in which, at lower doses, it acts as a stimulant and at higher doses, it portrays sedative effects (Kruegel and Grundmann, 2018). Therefore, it is not surprising that Kratom has traditionally been used for both its stimulant properties as well as an opium substitute (E Adkins, W Boyer and R McCurdy, 2011). At high doses, it is also shown to induce analgesic effects. Among Kratom's other uses are analgesia, for opioid withdrawal symptoms, and as a mood enhancer (Kruegel and Grundmann, 2018). In a study conducted in Malaysia, one study investigating the characteristics of regular kratom users involving 293 male users found that the users also viewed kratom as a replacement for alcohol and narcotics in cocktails (D Singh *et al.*, 2018).

A socio-demographic study performed among regular Kratom leaf users found that the main motives for its usage include positive reinforcement, negative reinforcement and externally generated positive reinforcement (Singh *et al.*, 2019). It is shown that heavy Kratom intake (> 3 glasses daily, each glass containing 48.24–50.4 mg of mitragynine) was associated with coping ($t_{87.09} = 3.544$, $p < 0.001$) and enhancement ($t_{114} = 2.180$, $p = 0.03$). Relative to married subjects, single subjects had higher mean scores in the coping domain (Singh *et al.*, 2019).

2.4 Kratom studies in human

There are very limited studies available that look into the effects of regular Kratom use on the brain structures, specifically assessed using Magnetic Resonance Imaging. There is one preliminary study of Brain Magnetic Resonance Imaging of regular Kratom users performed (involving seven regular kratom users and seven non-kratom users), which showed that long-term Kratom usage is not significantly associated with

altered brain structures, however, further study is needed to establish more data for kratom use and its effects (Darshan Singh *et al.*, 2018). Most of the Kratom related studies revolve around its pharmacological properties analysis, socio-demographics of regular Kratom users and behavioural effects of Kratom on its users.

Side-effects of Kratom have been reported in case reports (West) and not in surveys (South East Asia). In the West, clinical case reports that associate kratom use with adverse events or fatalities frequently pertained to kratom used together with other substances. Thus, it is important to evaluate these case reports with caution as it would be unfortunate to put legal sanction on Kratom based on this evidence alone as the experiences from South East Asia suggest considerable potential for therapeutic use among people who use drugs (Singh, Narayanan and Vicknasingam, 2016).

2.5 Potential effects of Kratom on brain

The active ingredients are alkaloid substances called mitragynine and 7-hydroxymitragynine, and are mediated via the monoaminergic and opioid (μ - and κ -) receptors (Warner, Kaufman and Grundmann, 2016). These mechanisms of action are similar to opioids partly due to their molecular structure, referred to as biased agonists (Henningfield, Fant and Wang, 2018). Kratom is classified as a receptor-biased agonist, which refers to its properties acting as a partial agonist at the μ -opioid receptor with a signalling bias for G-protein-mediated pathways without recruitment of beta-arrestin2 (Kruegel and Grundmann, 2018). A study has shown that there were no significant differences in the haematological and clinical-chemistry parameters between traditional kratom users and healthy controls (D Singh *et al.*, 2018). In a case report, Kratom has been shown to produce reversible injury to the posterior white matter of the

brain (Castillo, Payne and Nugent, 2017). A significantly limited number of studies are available in evaluating the effects of regular Kratom exposure on the human brain.

Effects of long term exposure on the human brain of other abused substances including opioids are more vastly studied. Two longitudinal studies on adults have suggested that opioids can induce structural brain changes (Younger *et al.*, 2011; Lin *et al.*, 2016), with volumetric changes in reward- and pain-related brain regions observed after 1 month of daily morphine administration in patients with chronic low back pain.

A study by Sirnes *et al* regarding brain morphology of children with prenatal opioid exposure revealed that volumes of basal ganglia, thalamus and cerebellar white matter were reduced in the opioid-exposed group while there were no significant differences in the global brain measures (Sirnes *et al.*, 2017). A neuroimaging meta-analysis in 2017 studying grey matter abnormalities in opioid-dependent patients found that the primary site of grey matter deficits is the bilateral frontotemporal region (Wollman *et al.*, 2017). The length of opioid use is negatively associated with grey matter in the right Rolandic operculum (including insula) and the left cerebellar vermis (Wollman *et al.*, 2017). Reduced volumes of the amygdala in prescription opioid-dependent patients and the nucleus accumbens in heroin-dependent patients have also been reported in cross-sectional studies (Upadhyay *et al.*, 2010; Seifert *et al.*, 2015). A study involving 16 neonates with prenatal opioid exposure demonstrated reduced total brain and basal ganglia volumes, compared to population values (Yuan *et al.*, 2014). In another study, it was found that there is altered white matter characteristics and a reduction in neuroanatomical volumes, particularly in the pallidum and putamen, in a group of ten school-aged children with prenatal exposure to opioids (Walhovd *et al.*, 2007, 2010)

Another study among subjects with polysubstance abuse showed that there is a significantly smaller grey matter volume of the prefrontal lobe (left and right hemispheres) in the substance abuse group compared to the control group (Liu *et al.*, 1998). Likewise, another structural MRI study among substance abusers (intravenous cocaine, methamphetamine, intravenous heroin and cannabis) has shown that there is a significantly less frontal white-matter volume percentage among substance abusers compared to the controls group (Schlaepfer *et al.*, 2006).

The cortico-basal ganglia-thalamic circuit has long been implicated in the pathophysiology of addictive behaviours (Yager *et al.*, 2015; Loonen, Schellekens and Ivanova, 2016). The striatum in particular, is the major integration site of this complex circuit and play an important role as part of the reward circuit (Yoo *et al.*, 2021). The striatum can be divided into two major sites, the dorsal striatum (which consist of caudate nucleus and putamen) and the nucleus accumbens. Some animal studies have also implicated caudate nucleus and putamen in drug addiction seeking behaviour (Hearing, Schwendt and McGinty, 2011).

In Malaysia, the cultivation of kratom trees is legal, but the possession, distribution, and processing of kratom are not, under the Poisons Act 1952. It is prohibited in Malaysia due to its alleged addictive properties. Despite the reported health incidents, kratom remains unregulated at the Federal level in the U.S, although the Food and Drug Administration (FDA) has claimed kratom to be “an opioid” (Singh *et al.*, 2019).

2.6 Conceptual Framework

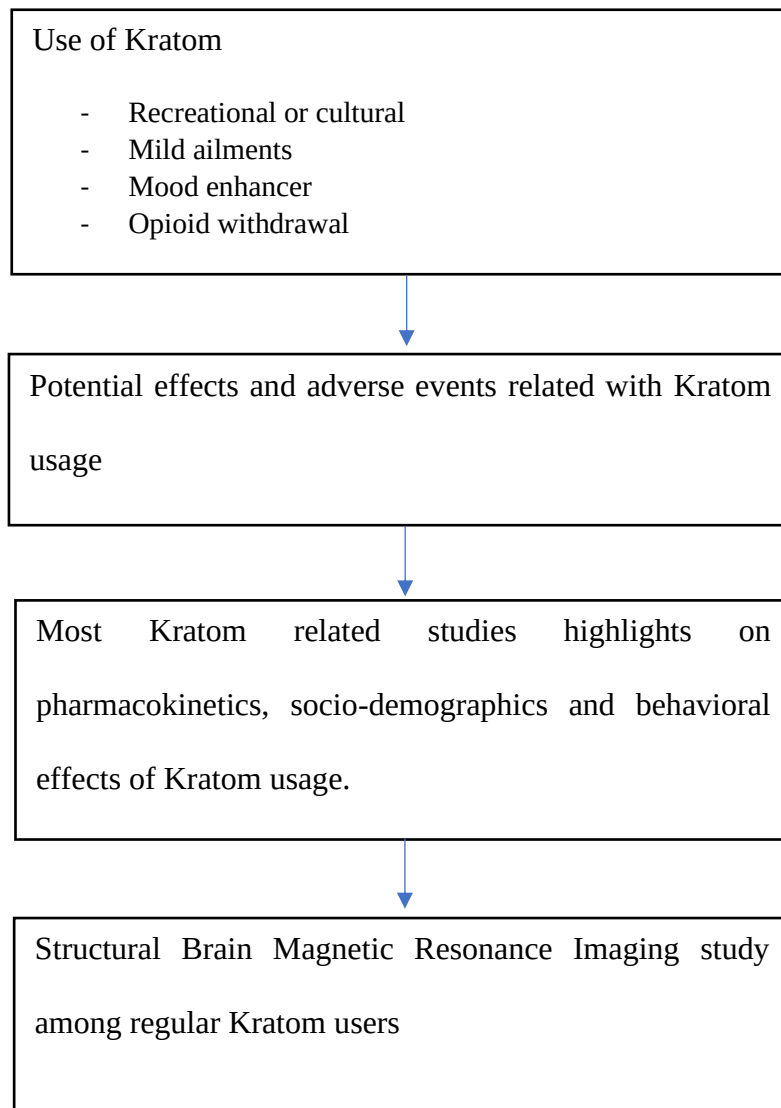


Figure 6: Conceptual framework

2.7 Study Rationale

Kratom (*Mitragyna speciosa*, Korth) has been widely used in Southeast Asia for centuries especially among the rural population for multiple purposes (Singh *et al.*, 2019). There is increasing use of Kratom which is not only limited to Southeast Asia but globally likely due to market globalization and readily available internet-driven supply chains (Tatum *et al.*, 2018). Despite the increasing usage of Kratom, there is a very limited number of studies that are currently available that explore the effects of regular Kratom usage on the brain.

Most of the Kratom-related studies has their focus on the epidemiology and behavioural characteristics of regular Kratom users. To our knowledge, despite the long history and increasing usage of Kratom, there is only one preliminary study that explores the effects of regular Kratom leaves user on the neuroanatomical structures of the brain (Darshan Singh *et al.*, 2018).

It is hoped that this study will add useful information to the limited knowledge that is available concerning the effects of long term Kratom use on the brain. The data from this study may help in future research related to Kratom and exploration of its potential beneficial pharmacological properties to aid in our future medical treatment. Conducting this study may also contribute to the level of the investigator's professional development.

CHAPTER 3: METHODOLOGY

3.1 Research Design

This will be a retrospective cross-sectional study involving regular adult Kratom leaves users who underwent MRI Brain at Radiology Department, Advance Medical and Dental Institute, Penang for another research (No: 304.CIPPT.6315186). For the controls, it will involve healthy subjects who underwent MRI Brain as part of the normal database at the Radiology Department, Hospital Universiti Sains Malaysia.

3.2 Study area and study population:

3.2.1 Kratom users: Regular Kratom users in the state of Penang who underwent MRI Brain for another research (No: 304.CIPPT.6315186).

3.2.2 Non-Kratom users: Previously recruited healthy volunteers (age and gender-matched to non-control subjects) who underwent MRI Brain as part of normal database at Radiology Department, Hospital Universiti Sains Malaysia (HUSM), Kubang Kerian, Kelantan.

3.3 Subject criteria

3.3.1 Inclusion criteria:

1. Age ≥ 18 years and less than 60 years old.
2. Must be a regular kratom user (used kratom ≥ 1 time daily) with >6 months of kratom use (Singh, Müller and Vicknasingam, 2014).

3.3.2 Exclusion criteria:

1. With a previous and current history of neurological and psychiatric disorders.
2. Previous and current drug and alcohol dependence history, who tested positive for illicit drug use such as (opiate, cannabis, methamphetamine, amphetamine, methadone, benzodiazepine and ketamine).
3. History of significant brain injury or concussion.

Healthy controls are also subjected to the same inclusion and exclusion criteria, except they must not have a kratom use history.

3.4 Sampling method

A convenience sampling technique will be used for this study. MRI Brain images of Kratom users and non-Kratom users taken from a database of previous research which fulfils all the inclusion and exclusion criteria will be included in this study. The data collection will be done by taking subjects' images from the PACS system into Osirix image viewer version 3.2.1. (or Horos image viewer version 3.3.5.) for further data analysis.

3.5 Sample size calculation

1. To determine the mean brain volumes (total intracranial volume, total brain volume, volumes of caudate nucleus and putamen) among regular adult Kratom leaves users.

Based on an article by (Sirnes *et al.*, 2017) prior data indicate that the mean intracranial volume was 1521.48 (standard deviation = 148.23). Sample size estimation is calculated by Power and Sample size calculator (Dupont and Plummer, 1990) using the population mean formula. We are planning a study of a continuous response variable from independent control and experimental subjects with 1 control(s) per experimental subject. In a previous study, the response within each subject group was normally distributed with a standard deviation 148.23. If the true difference in the experimental and control means is 103.77, we will need to study 33 experimental subjects and 33 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with the probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05. With an additional 10% dropout rate, the sample size is 37 samples.

Survival | t-test | Regression 1 | Regression 2 | Dichotomous | Mantel-Haenszel | Log

[Studies that are analyzed by t-tests](#)

Output

[What do you want to know?](#) Sample size

[Sample Size](#) 33

Design

[Paired or independent?](#) Independent

Input

[α](#) 0.05 [δ](#) 103.77 [Calculate](#)

[σ](#) 148.23 [Graphs](#)

[power](#) 0.8 [m](#) 1

(Dupont WD, 1990)