NEUROPROTECTIVE EFFECTS OF XANTHONE-ENRICHED FRACTION *GARCINIA MANGOSTANA* AND ALPHA-MANGOSTIN IN CHRONIC CEREBRAL HYPOPERFUSION RATS

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by

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this dissertation can provide useful information for the future students and supervisor for investigation of new knowledge.

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LIST OF SYMBOLS AND ABBREVATIONS

%	Percentage sign
μg	Microgram
μL	Microliter
μm	Micrometer
α-MG	Alpha-mangostin
2VO	Two-vessel occlusion
ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's disease
AKT	serine/threonine kinase
AKT	Protein kinase B
ALP	Alkaline phosphatases
ALT	Alanine transaminase
AMPA	Alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ANOVA	Analysis of variance
AP	Anterior-posterior
APS	Ammonium persulphate
APV	Amino phosphono valeric acid
ARASC	Animal Service and Research Centre
AST	Aspartate transaminase
Aβ	beta amyloid
BCAS	Bilateral common carotid artery stenosis
BDNF	Brain-derived neurotrophic factor
BSA	Bovine serum albumin
BuChE	Butytrylcholinesterase
CA1	Cornu ammonis 1

Ca^{2+}	Calcium ion
CA3	Cornu ammonis 3
CaM	Ca^{2+}/Calmodulin
CGNs	Cerebellar granule cultured neurons
CGNs	Cerebellar granule cultured neurons
ChAT	Choline acetyltranferase
ChE	Cholinesterase
CREB	Cyclic AMP response element binding protein
CYFIP1	Cytoplasmic FMR1-interacting protein
DNA	Deoxyribonucleic acid
DTNB	5,5'-Dithiobis (2-nitrobenzoic acid)
DTT	Dl-dithiothreitol
Elk-1	ETS Like-1 protein
e-LTP	Early long-term plasticity
ERK	Extracellular-signal-regulated kinase
fEPSPs	Field excitatory postsynaptic potentials
FRS-2	Fibroblast growth factor receptor substrate 2
FMRP	Fragile X mental retardation
g/kg	Gram per kilogram
GABA	Gamma-aminobutyric acid
h	Hour
H_2O	Water
H_2O_2	Hydrogen peroxide
Hb	Hemogloblin
HCl	Hydrochloric acid
HFS	High frequency stimulation
HRP	Horseradish peroxidase

Hz	Hertz
i.p	Intraperitoneal
I/O	Input/output
IC_{50}	Half maximal inhibitory concentration
IP3	Inositol trisphosphate
kDa	Kilodalton
LDS	Lithium dodecyl sulfate
IL-8	Interleukin-8
l-LTP	Late long-term plasticity
LTD	Long-term depression
LTP	Long-term potentiation
M	Molar
mA	Milliampere
MAPK	Mitogen-activated protein kinase
MCAO	Middle cerebral artery occlusion
mg/kg	Milligram per kilogram
Mg^{+}	Magnesium ion
$MgCl_2$	Magnesium chloride
ML	Middle-lateral
mM	Milli molar
mm	Millimeter
MNK	MAPK interacting protein kinases
ms	Milliseconds
mTORC1	Mammalian target of rapamycin complex 1
mV	Millivolts
MWM	Morris water maze

Na^+	Sodium ion
nAChR	Nicotinic acetycholine receptor
nm	Nanometer
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
OD	Optical density
OFT	Open Field Test
p	Probability of neurotransmitter release
PEG	Polyethylene glycol
pH	Power of hydrogern
PI3K	Phosphoinositide-3-kinase
PKC	Protein kinase C
PLC	Phospholipase C
PLT	Platelet
PPF	Paired-pulse facilitation
PSD	Postsynaptic density
PTP	Post-tetanic potentation
ROS	Reactive oxygen species
S.E.M	Standard error mean
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
STSP	Short term synaptic plasticity
TBS	Theta-burst stimulation
TBST	TRIS-buffered saline contained 0.05% v/v tween 20
TEMED	N, N, N', N'- Tetramethylethylenediamine
TIA	Transient ischemic attack

TNB	2-nitro-5thiobenzoate
TNF-α	Tumor necrosis factor alpha
Trk	Tyrosine receptor kinase
TrkB	Tropomyosin receptor kinase B
TrkB-Fc	Fusion of tropomyosin receptor kinase B and fc domain of human IgG
V	Ventral
v/v	Volume per volume
VaD	Vascular dementia
VCI	Vascular cognitive impairment
w/v	Weight per volume
WBC	White blood cells
XEFGM	Xanthone enriched fraction of G*arcinia. mangostana*

KESAN PELINDUNGAN SARAF OLEH FRAKSI YANG KEKAYAAN DENGAN XANTON DARIPADA *GARCINIA MANGOSTANA* DAN *ALPHA-MANGOSTIN* DALAM TIKUS SEREBRUM HIPOPERFUSI KRONIK

ABSTRAK

Demensia vaskular (VaD) adalah penyakit sistem saraf yang teruk; ianya menyumbang kepada 15-20% daripada kes-kes demensia yang sering disebabkan oleh gangguan saluran darah ke otak. Faktor utama risiko muncul dari hipertensi, kencing manis, serangan iskemia sementara (TIA), strok atau peyakit kardiovaskular yang lain. Kadar kejadian VaD ini dianggarkan berlaku pada 6-12 orang dalam 1000 individu di seluruh dunia setiap tahun selama 70 tahun. Xanthon yang dipencil dari perikarpa *G. mangostana* dan sebatian utamanya α-mangostin (α-MG) telah dilapor menunjukkan kesan pelindungan saraf secara *in vitro* dan *in vivo*. Dalam kajian ini, kesan perlindungan zat-zat diperkaya xanthon daripada *G. mangostana* (XEFGM) dan α-MG dinilai dalam model tikus serebrum hipoperfusi kronik atau dikenali sebagai oklusi dua saluran darah (2VO). Pada bahagian pertama kajian ini, XEFGM (25, 50, dan 100 mg/kg) dan α-MG (25 dan 50 mg/kg) diberikan kepada tikus 2VO dan ditaklukkan kepada ujian tingkahlaku bagi menilai fungsi pembelajaran dan memori rujukan. Hasil kajian mencadangkan bahawa, 50 mg/kg α-MG yang diberikan kepada tikus 2VO menambahbaik memori spasial dan memori rujukan secara signifikan dalam ujian berselirat air. Tisu korteks hadapan, hippokampus, korteks serebral daripada tikus tersebut dipencilkan bagi analisis asetilkolinesterase (AChE) dan butitrilkolinesterase (BuChE). Hasil kajian menunjukkan, rawatan dengan α-MG (50 mg/kg) dalam model 2VO meningkatkan aktiviti AChE secara signifikan dalam hippokampus tikus 2VO. Hasil kajian ini membawa kepada bahagian

kedua kajian dimana 50 mg/kg α-MG diberikan kepada tikus 2VO selama 14 hari berturut-turut. Tikus 2VO yang dirawat tersebut dinilai pembelajaran dan fungsi memori serta mekanisma yang mungkin terlibat dalam meningkatkan fungsi kognitif. Penurunan aktiviti AChE dalam hippokampus pada tikus 2VO dan rawatan α-MG selama 14 hari memulihkan aktiviti AChE telah dilaporkan dalam kajian ini. Manakala penurunan signifikan aktiviti BuChE dapat dilihat didalam korteks serebral tikus 2VO. Tambahan pula, rakaman elektrofisiologi secara *in vivo* menyatakan bahawa 2VO secara signifikan melemahkan kekuatan sinaps pada CA3-CA1 dalam kawasan hippokampus dan mengurangkan kadar ekspresi protein faktor neurotropik yang berasal dari otak (BDNF) yang mana merupakan pengawal selia utama dalam menggalakan potentiasi jangka panjang (LTP). Walau bagaimanapun, rawatan selama 14 hari α-MG dilihat tidak mempunyai kesan kepada kekuatan sinaps pada CA3-CA1 dalam kawasan hippokampus dan ekspresi protein BDNF di dalam hippokampus. Akhir sekali, α-MG didapati selamat untuk digunakan kerana tidak ada kesan ketoksikan yang dilihat selepas pengambilan secara oral selama 14 hari. Secara keseluruhan, kajian ini mencadangkan bahawa α-MG memperbaiki pembelajaran dan deficit memori dalam model 2VO dengan mengenakan kesan keatas aktiviti AChE didalam hippokampus. Oleh itu, α-MG mampu menjadi agen terapi yang mempunyai potensi keatas penyakit neurodegeneratif yang terkait dengan serebrum hipoperfusi kronik, termasuk demensia vascular dan penyakit Alzheimer.

NEUROPROTECTIVE EFFECTS OF XANTHONE-ENRICHED FRACTION OF *GARCINIA MANGOSTANA* (XEFGM) AND ALPHA-MAGOSTIN IN CHRONIC CEREBRAL HYPOPERFUSION RATS

ABSTRACT

Vascular dementia (VaD) is a severe neurodegenerative disorder; it accounts for 15-20% of dementia cases which is often caused by interruption of blood flow to the brain. The leading risk factors VaD are from hypertension, diabetes mellitus, transient ischemic attack (TIA), stroke or other cardiovascular diseases. The incidence of VaD is estimated to occur at 6-12 in every 1000 individual worldwide per year for 70 years. Xanthone isolated from pericarp of *G. mangostana* and its major compound α-mangostin (α-MG) have been reported to exhibit neuroprotective effect *in vitro* and *in vivo.* In this study, the protective effect of xanthone enriched fraction of *G. mangostana* (XEFGM) and α-MG were evaluated in an animal model of VaD induced by chronic cerebral hypoperfusion also known as- two vessel occlusion (2VO). In the first part of this study, XEFGM (25, 50, and 100 mg/kg) and α-MG (25 and 50 mg/kg) were administrated to 2VO rats and the rats were then subjected to behavioral test for learning and reference memory evaluation. The result suggested that, 50 mg/kg of α-MG treated 2VO significantly improved spatial learning and reference memory in Morris water maze test. The frontal cortex, hippocampal and cerebral cortex tissues from the rats were extracted for acetylcholinesterase (AChE) and butytrylcholinesterase (BuChE) activity. The results revealed that, treatment with α-MG (50 mg/kg) in 2VO model significantly increased the AChE activity in hippocampus of 2VO. These results lead to the second part of this study where 50 mg/kg of α-MG were administrated in 2VO rats for 14 consecutive days. The 14 days α-MG treated 2VO rats

were assessed for their learning and memory function and its possible underlying mechanisms involve in improving cognitive function. We reported suppression of AChE activity in 2VO hippocampal region and 14 days 50 mg/kg α-MG treatment did not cause significant changes in 2VO AChE activities. While a significant suppression of BuChE activities were observed in cerebral cortex of 2VO rats but no effect with 14 days α-MG treatment. Furthermore, *in vivo* electrophysiological recording demonstrated that 2VO significantly attenuated synaptic strength in CA3-CA1 of hippocampal region and downregulated the expression of hippocampal brain-derived neurotrophic factor (BDNF) protein which is a key regulator in promote long-term potentiation (LTP). However, 14 days treatment of α-MG found to has no effect on the hippocampal CA3-CA1 synaptic plasticity as well as on the expression of BDNF protein in hippocampus. Lastly, α-MG found to be safe for consume as no toxicity sign were observed after 14 days of orally administration of α-MG. Overall, this study suggest that α-MG ameliorated learning and memory deficits in 2VO, the decreased level of AChE in hippocampus asscociated with 2VO were significantly prevented by acute administration of α-MG. Therefore, α-MG could be a promising therapeutic agent for CCH associated neurodegenerative diseases, including vascular dementia and Alzheimer's disease.

CHAPTER 1

INTRODUCTION

Dementia is commonly recognized as memory loss or a decline in cognitive abilities that disturb the patient's emotional and daily executive activities (Farid et al., 2012). It consist a cluster of symptoms which including anxiety, psychosis, depression, apathy, dysphoria, agitation, aberrant motor behavior, eating disorder as well as sleep disturbances (Van Der Linde et al., 2014). Vascular dementia (VaD) is one of the most common causes of dementia after Alzheimer's disease (AD) which usually develops in older people. Although, VaD and AD are two independent diseases that arises from different etiology but they do share common pathological features such as reduced cerebral blood flow (CBF) to the brain which have been identified in both VaD and AD patients. The reduced CBF, also known as cerebral hypoperfusion, is thought to be one of the major factor leads to cognitive impairment (Sarti et al., 2002) .

Cerebral hypoperfusion causes a series of neuropathological cascades in brain such as depletion of glucose and mitochondria, production of reactive oxygen species (ROS), apoptosis of pyramidal neuron in hippocampus, activation of microglial cells, deformation of the protein-involved neurotransmission in the brain and white matter lesion, these are the pathological changes that result in cognitive impairment (Somredngan et al., 2017; Liu et al., 2012). In order to mimic human chronic cerebral hypoperfusion (CCH) in animal model, permanent bilateral occlusion of common carotid arteries (2VO) in rats is the most suitable model for CCH study due to their poor cognitive performance in several

behavioral task as well as the alteration of molecular and cellular signaling in brain (Damodaran et al., 2014; Farkas et al., 2007). Moreover, this model has been widely used to investigate the effect of potential neuroprotective agents to the CCH associated with neurodegenerative diseases in several studies (Damodaran et al., 2018; Kim et al., 2016; Xu et al., 2010). These studies demonstrated that, treatment of drug with properties of antioxidant, anti-inflammation and increase acetylcholine level in the brain can delay or prevent the progression of 2VO induced CCH associated learning and memory deficits.

G. mangostana or mangosteen is a tropical fruit tree that mainly grows in Southeast Asia. The fruit of *G. mangostana* is white and juicy and it has sweet and sour taste. Over decades, the purple pericarp of *G. mangostana* has been used as traditional medicine for treating diarrhea, trauma, skin infection and wound (Jung et al., 2006). Furthermore, the pericarp contains rich source of prenylated xanthone which is a kind of secondary metabolites and their major constituent was found to be α-mangostin (α-MG). The *G. mangostana* pericarp extract and this compound was proven to exhibit a wide range of pharmacological effects including anti-diabetic, anti-oxidant, anti-inflammation, anti-tumour, anti-bacteria and the most intriguing activity is their neuroprotective effects (Ibrahim et al., 2015). In 2014, Murugaiyah and his colleague found that α-MG was a promising AChE inhibitor (Murugaiyah et al., 2014). At present, one of the treatments for AD and VaD patients is the dispensation of AChE inhibitors like donezepil and tacrine that increase the availibity of acetylcholine at the cholinergic synapses (Pandareesh et al., 2016). Hence, the focus of the study is to determine new potential AChE inhibitor replacing the current drugs with adverse effects. Furthermore, several studies reported that α-MG showed neuroprotective effect in various neuronal cell lines was due to their strong

ROS scavenging activity (Weecharangsan et al., 2006; Pedraza-Chaverrí et al., 2009; ; Beltran et al., 2008). While, there is limited finding to demonstrate effects of *G. mangostana* and its main compound, α-MG on *in vivo* studies. Mangosteen extract has been shown to have protective effects against oxidative stress, inflammation (Jung et al., 2006b, Chen et al., 2008) and recent studies showed that mangosteen pericarp extract reverse the cholinergic dysfunction induced by lead (Phyu et al., 2014). From the accumulating evidences has indicated that CCH associated cognitive impairment could also be reversed when treated with mangosteen pericarp and α-MG. Although the protective effects of Garcinia mangostana on memory impairment have been reported, the molecular player and the process leading to the improvement of memory in CCH *in vivo* have not been described. Therefore, in this study is to justify the potential effects of XEFGM and α-MG treatments in learning and memory function of CCH model, to determine the effects of XEFGM and α-MG on acetylcholinesterase and butytrylcholinesterase activities in brain of 2VO model and to determine the possible underlying mechanism that XEFGM and α-MG treatments could modulate in the learning and memory function of 2VO model.

1.1 Objectives:

In the present study, we used xanthone enriched fraction of *G. mangostana* (XEFGM) and α-MG to investigate their impact on the learning and memory of CCH model. In order to accomplish the aim of this study, the following objectives were conducted:

A. Effect of acute administration of XEFGM /α-MG on the cognitive functions and acetylcholinesterase/butytrylcholinesterase avtivities in 2VO rats.

1. To evaluate the protective effect of XEFGM /α-MG in the spatial learning and reference memory in 2VO rats using Morris water maze test.
2. To investigate the protective effects of XEFGM /α-MG on acetylcholinesterase (AChE)/ butytyrlcholinesterase (BuChE) in different brain regions (frontal cortex, hippocampus, and cerebral cortex) in 2VO rats.

B. Effect of 14 days administration of XEFGM/ α-MG on the learning and memory function and the underlying mechanism in 2VO rats.

- To evaluate the protective effect of 14 days administration of XEFGM/α-MG in the spatial learning and reference memory in 2VO rats using Morris water maze test.
- To investigate the protective effects of 14 days administration of XEFGM/α-MG on acetylcholinesterase (AChE)/ butyrylcholinesterase (BuChE) in different brain regions (frontal cortex, hippocampus, and cerebral cortex) in 2VO rats.
- To determine the protective effects of 14 days administration of XEFGM/α-MG on synaptic plasticity in CA3-CA1 region of hippocampus of 2VO rats using *in vivo* electrophysiological recording.
- To investigate the protective effects of 14 days administration of XEFGM/α-MG on expression level of synaptic proteins brain-derived neurotrophic factor (BDNF) and Ca^{2+}/calmodulin dependent protein kinase (CaMKII) in the hippocampus of 2VO rats.

- To determine the toxicity effects of 14 days of XEFGM/α-MG administration in 2VO rats.

To the best extent of our knowledge, the present study is the first to provide useful mechanistic information on the effect of mangosteen extract and α-MG on the 2VO induced CCH models.

Neuroprotective effect of xanthone-enriched fraction *G. mangostana* (XEFGM) and α-mangostin (α-MG) for the treatment of memory disorder and improves cognitive behavior in chronic cerebral hypoperfusion rats.

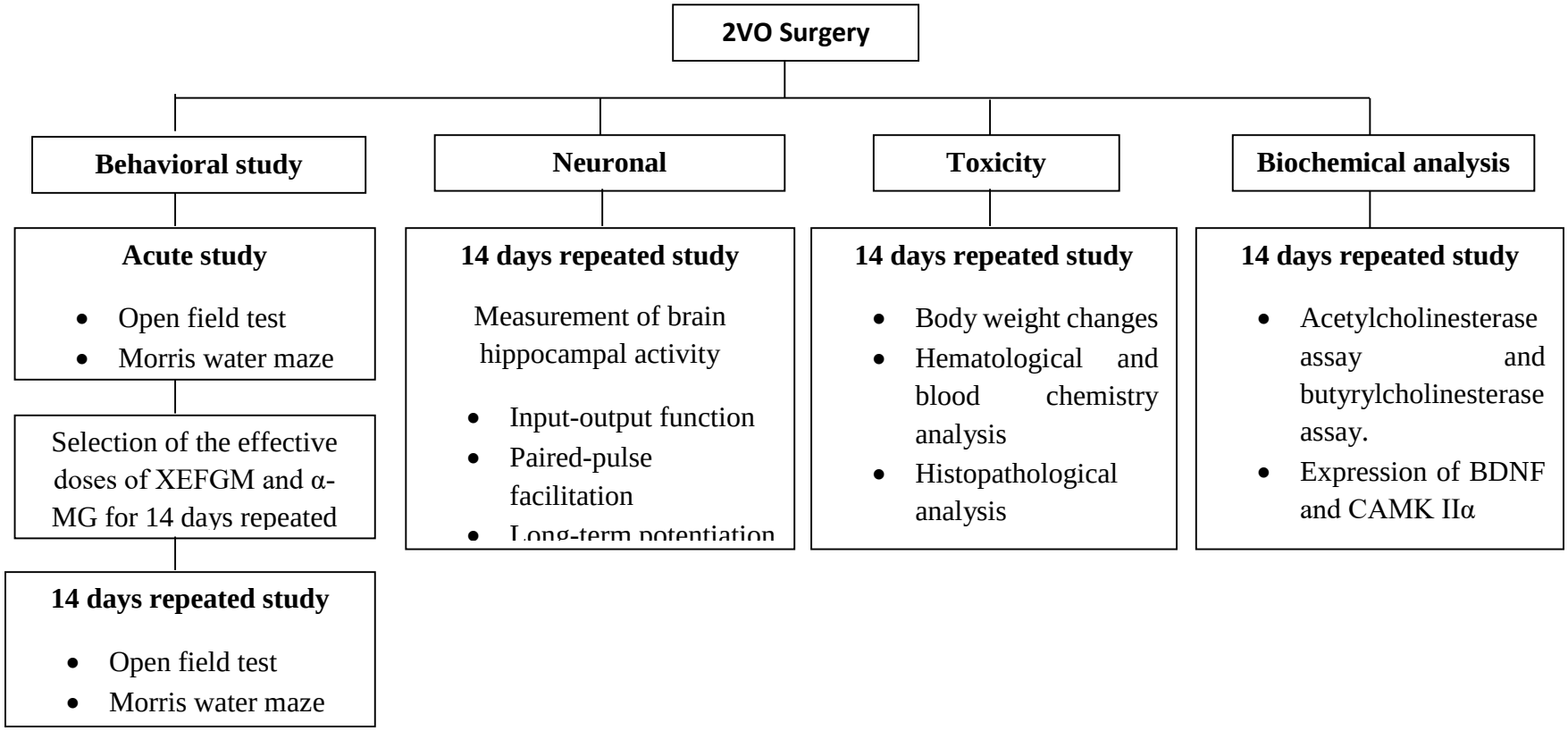

Figure 1.1: General outline of the study on the effect of XEFGM and α-MG on cognitive behavior and brain activity.

CHAPTER 2

LITERATURE REVIEW

2.1 Traditional medicinal uses of G. mangostana

For the longest time, humankind have tried to use tropical plant as a medication to cure various illness and general well-being. The healing benefits of a certain plant were conveyed from person to person and to the successive generation. Unfortunately, there is limited scientific evidence of their medicinal value and safety consumption of most herbal products. On the other hand, the continuation of deforestation occurred around the world could endanger potential therapeutically plants before they are discovered and examined by the scientists. Hence, it is important for the scientists to actively examine chemical properties of the plant before it gets in danger of extinction. One nearly miss event happened decades ago with a compound- Calanolide A , a derivatives from *Calophyllum lanigerum var austrocoriaceum*, belongs to the Guttiferae or mangosteen family has shown significant inhibitory effects against human immunodeficiency virus (HIV) (Kashman et al., 1992) was almost wiped out. The plant samples of *Calophyllum lanigerum var austrocoriaceum* were first collected at Kerangas forest in Sarawak, Malaysia in 1987. Unfortunately, after the plant was identified with anti-HIV effects, the scientists returned to the following locations, the plant disappear due to logging activities (Butler, 2005).

G. mangostana is also a member of Guttifrae family. It is a tree that are grown in the tropical rainforest of Southeast Asia such as Malaysia, Indonesia, Thailand and

Philippines (Pedraza-Chaverri et al., 2008). The fruit produced by *G. mangostana* is referred as mangosteen. For the natives of these countries, the pericarp (skin, peel or hull) of the fruit, leaves and bark of the tree could be apply externally as an ointment for treating wound and skin disorder like eczema, psoriasis and hyperkerotosis (Obolskiy et al., 2009). Studies have shown that, these parts of the mangosteen trees contain strong anti-inflammatory properties thus it could be useful in treating skin infection (Chomnawang et al., 2007; Widowati et al., 2016). The pericarp is famous for orally consumption to relieve diarrhea, gonorrhea, cystitis and gleet (Obolskiy et al., 2009). It is believed that the mangosteen pericarp is very nutritious and it could replace the loss of essential nutrient from the body due to dehydration.

In Malaysia, Indonesia and Philipinnes, mangosteen leaves and barks are grounded into powder to make tea and it is commercially available as dietary supplement product to lower blood sugar level (Udani et al., 2009) and in treating urinary tract infection (El-Kenawy et al., 2011). Furthermore, decoctions of the roots have been administered by women for regulating menstrual cycle (Obolskiy et al., 2009). Other than Southeast Asia, mangosteen fruit juices and tea are popular in Latin America and Caribbean, the tea is known to enhance body strength for people who are lack of energy or fatigue In Brazil, people use mangosteen tea as a digestive aids (Obolskiy et al., 2009).

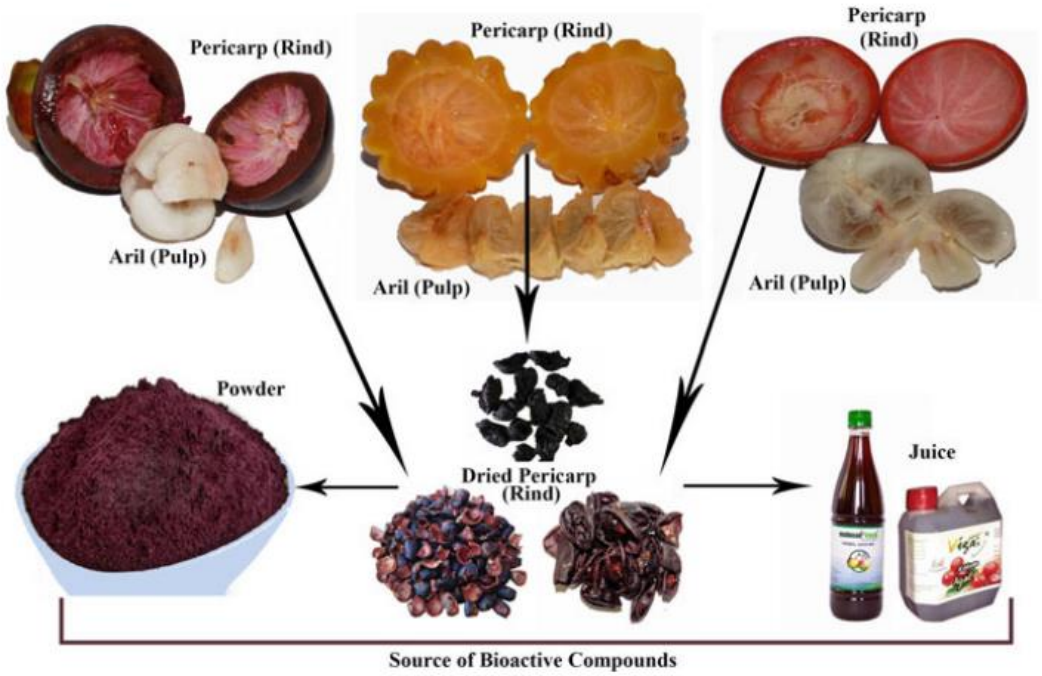

Figure 2.1: Fruits, dried pricarp (rind), powdered pericarp and juice of mangosteen, used as a source of bioactive compounds (adapted from Murthy et al., 2019).

2.2 Chemical structure and pharmacological properties of *G. mangostana*

The main bioactive component in G. mangostana consists plenty of prenylated xanthone derivatives, they are secondary metabolites which produced in fungi, lichen, bacteria and plants (Obolskiy et al., 2009). The functional groups of xanthone comprise of phenyl group, isoprene, hydroxyl and aromatic protons, methoxy, phenolic hydroxyl group as well as dihydrofuran rings (Shan et al., 2011). These several different substituents arrange themselves into tricyclic aromatic ring which made up of the skeleton structure of a xanthone (El-Kenawy et al., 2011). The basic xanthone structure is determined by 1H and 13C nuclear magnetic resonance (NMR) (Suksamrarn et al., 2006) and over 60 different types of xanthone have been recognized from various part of G. mangostana plants, such as α-mangostin, β-mangostin, γ-mangostin, mangostenones C,

mangostenones D, mangostenones E, garcinone B, garcinone C, garcinone D, garcinone E, 8-desoxygartanin, gartanin (Suksamrarn et al., 2006; Jung et al., 2006). The major xanthone compound, α-mangostin which often obtained as a yellow-colored and it was found the most abundant from the pericarp of mangosteen (Ibrahim et al., 2016). This compound has been well studied and known to exhibit some significant biological actions:

2.2.1 Anti-inflammatory properties

The anti-inflammatory actions in *in vivo* study was demonstrated by Chen et al. (2008), treatment of α-mangostin showed significant inhibition of carrageenan-induced paw edema in mice at 3h and 5h after carragenan administration. The study also showed both xanthone α-mangostin and ϒ-mangostin markedly diminished lipopolysaccharide (LPS) stimulated nitric oxide (NO) and PGE_2 productions in RAW 264.7 cells with the IC_{50} values of 12.4 and 10.1 μM, respectively. Furthermore, Gutierrez-Orozco et al. (2013) showed *α-mangostin* suppressed the secretion of the inflammatory mediators TNF-α and IL-8 in various tissue origins of human cells. Furthermore, increasing evidence shows that plants exhibit anti-oxidative or anti-inflammation properties has beneficial effect on the cognitive memory of CCH model. Huperzine A from species *Huperzia serrata*, (Wang et al., 2010), Fructus mume extract (Jeon et al., 2012), *Ginkgo bibola L*. extract (Kim et al., 2016), Morin from *Maclura cochinchinensis* that belongs to Moracae family (Khamchai et al., 2020) have shown to improve cognitive impairment induced by CCH through anti-inflammation pathway.

2.2.2 Neuroprotective properties

Compound and extract of *G. mangostana* has been widely reported to have neuroprotective effect in the recent years (Hao et al., 2017; Wang et al., 2016; Janhom et al., 2015; Suksan et al., 2015; Phyu et al., 2014). Most of these wholesome effects of *G. mangostana* are believed to be strongly associated with their anti-inflammation and strong oxygen scavenging activities which can inhibit oxidative damage in tissue caused by various diseases. For example, Pedraza-Chaverr íet al. (2009) reported that α-mangostin able to scavenge several ROS directly and inhibit neurotoxicity by against 3-nitropropionic acid induced ROS in cerebellar granule cultured neurons (CGNs) in concentration dependent manner. In addition, the effects of antioxidant properties in neuroprotective activity were further proven in ethanol and water extract of *G. mangostana* when tested on hydrogen peroxide (H_2O_2) induced NG108-15 neuroblastoma cell death (Weecharangsan et al., 2006).

2.2.3 Anti-tumor properties

Multiple evidences from *in vitro* and *in vivo* have shown that xanthone isolated from *G. mangostana* inhibit human tumor cell proliferation via various signal transduction pathways. (Shan et al., 2011). Akao et al. (2008) found the ability of α-mangostin to induce intrinsic apoptotic pathway and suppress the signaling pathway through Mitogen-activated protein kinase (MAPK) and serine/threonine kinase (AKT) in human colorectal adenocarcinoma (DLD-1) cell lines. Panaxanthone contains at most 85% α-mangostin and 15% γ-mangostin suppressed mammary tumor growth and metastasis *in vivo* (Doi et al., 2009). Another study also showed that α-mangostin presents a significant inhibition on the growth of human prostate carcinoma by inducing apoptosis and inhibit the growth of

tumor in athymic nude mice (Johnson et al., 2012). Anti-diabetic (Nelli et al., 2013), anti-micbrobial (Nguyen & Marquis, 2011), antimalarial (Obolskiy et al., 2009), anti-obesity (Liu et al., 2015).

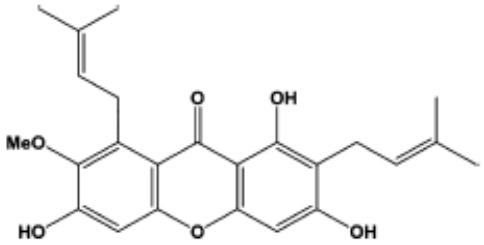

Figure 2.2: Structure of α-mangostin. (Adapted from Gutierrez-Orozco et al., 2013)

2.3 Dementia

Vascular cognitive impairment (VCI) is currently describe as a kind of syndrome diagnosed with stroke clinically or vascular injury in brain subclinically that affecting one or more brain cognitive regions and resulting in cognitive decline (Iadecola, 2013). VaD is the most severe type of VCI, accounting for more than 20% of dementia cases which is the second major leading cause of dementia follow after Alzheimer's diseases (Gorelick et al., 2016). During 1900s, hardening of arteries (arteriosclerosis) were believed to be the predominant cause of dementia for almost a decade, until discovery of beta amyloid (Aβ) peptides deposition forms amyloid plaque and amyloid angiopathy in various brain region which introduce "Aβ hypothesis" to Alzheimer's disease (Iadecola, 2013). Thereafter, attention on VaD was moved to AD but the significant effect of cerebrovascular distortion to dementia is inevitable. Previous clinical study revealed that, most of the dementia cases have comorbidities of AD features such as amyloid plaque and neurofibrillary tangles were associated with vascular lesions (Launer et al., 2008). Vascular dementia arises from

different and discrete ischemic injury (multi-infarct dementia) that leads to multiple stroke which frequently occur in patients with hypertensions as well as white matter lesions were often co-observed in the brain of dementia patients. Hence, treatment of vascular disease might lead to prevention of dementia (Hachinski et al., 1974).

The integrity of structural and function of cerebral vascular system are important for sustaining normal brain function. Although, human brain only occupied 2% of total body weight yet it has the highest energy and glucose consumption in human order to carry out neuronal activity such as restore and regulate the ionic gradient utilized for synaptic activity, and glucose is needed for the systhesis of brain neurotransmitter such as glutamate, acetylcholine and GABA (Magistretti & Allaman, 2012). Therefore, continuous supply and good blood circulation to the brain is essential to meet its high energy and oxygen demand.

2.4 Cerebral blood circulation network

The circle of Willis is a group of arteries that supply blood to the brain; it is basically constructed from two main arteries: internal carotid arteries and vertebral arteries. Internal carotid arteries emerge from the division of common carotid arteries in the neck and branches out into two main cerebral arteries: anterior cerebral arteries and middle cerebral arteries, both arteries are responsible for supply blood to the forebrain. For vertebral arteries, right and left vessel converged as midline basilar artery which intertwined with internal carotid artery and form into an arterial ring at the brain base, together with the merger of posterior cerebral arteries accompany with two small arteries: the anterior and posterior communicating arteries. This whole connecting network of

arteries forms the circle of Willis which provides an non interruptive supply of blood to the brain even when one of the artery is distrupted (Figure 2.2) (Purves et al., 2001).

The anterior and middle cerebral arteries from the circle of Willis are later divided into smaller branches that penetrate into deep structure region including basal ganglia, thalamus and internal capsule. While at the posterior part, cerebral, vertebral and basilar arteries supply blood to the midbrain and brain stem.

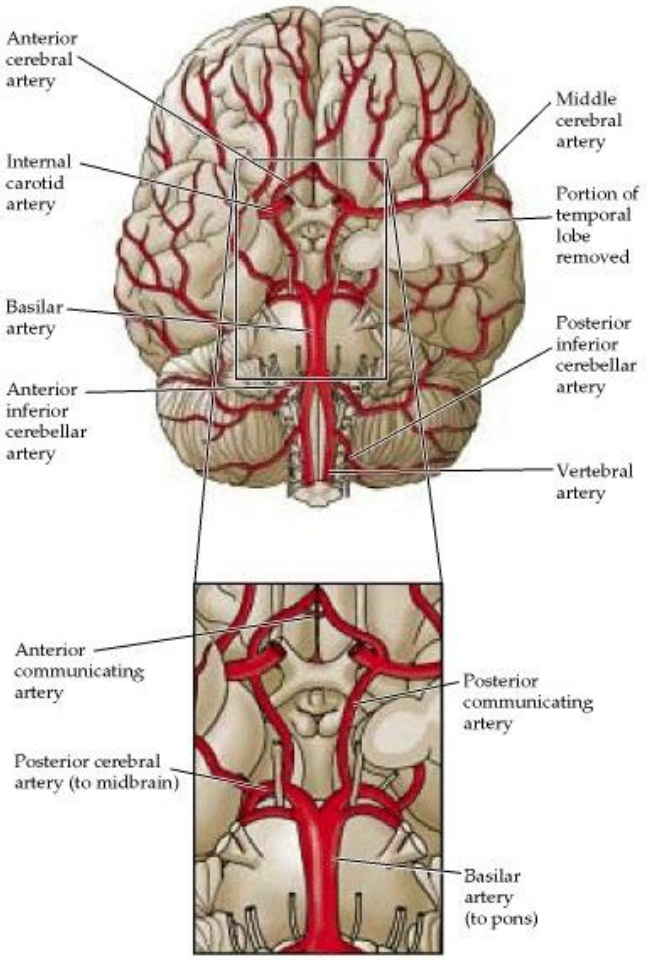

Figure 2.3: The network of arteries that provides blood supply to the brain. The circle of Willis was illustrated in the enlargement box above. (Adapted from Purves et al., 2001)

2.5 Chronic Cerebral Hypoperfusion (CCH) rodent model

Several experimental models have been introduced in rats to mimic CCH condition which occur in neurodegenerative diseases, including 2-vessel occlusion (2VO) (Du et al., 2017), middle cerebral artery occlusion (MCAO) (Back et al., 2017), bilateral common carotid artery stenosis (BCAS) (Shibata et al., 2004). Among all these models, 2VO model is the most common used model to represent CCH condition (Liu et al., 2012) as this model exhibits the prominent features of vascular dementia such as cognitive impairment and white matter injury. In addition, rats appear to represent the most suitable model for 2VO induction from other animals such as gerbils and mice (Farkas et al., 2007). Despite the reasons that rats are relatively cost effective and easily handle for behavioral testing (Bacigaluppi et al., 2010), they were chosen for 2VO surgery mostly due to their good survival and recovery rate after 2VO induction as their vascular structures comprises complete circle of Willis which allow blood flow compensation caused by ligation of common carotid arteries that lead to blood flow reduction instead of stroke (Farkas et al., 2007).

Studies of cerebral blood flow (CBF) in 2VO model have been carried out through hydrogen clearance (Ekl öf & Siesj ö, 1972; Fujishima et al 1981; Katayama et al., 1986) and autoradiographical approaches (Tsuchiya et al., 1992; Tsuchiya et al., 1993). CBF drops drastically after 2VO induction and remains low (average 30 to 45 % lower than the baseline) and this severe hypoxic ischemic condition occurs at the acute phase which usually lasts for 2 to 3 days after 2VO induction (Marosi et al., 2006). However, the blood flow gradually recover after 1 week of surgery but it is still markedly lowered up to 4 weeks after 2VO (chronic phase) (Farkas et al., 2007). 2VO triggered CBF reduction in

different brain region in several degree. CBF level in white matter area and cortical region is reduced up to 35-45%, while the most affected area is the hippocampus which up to 60% of CBF lower than the control (Ohta et al., 1997). The 2VO rats with significant CBF reduction exhibited severely impairment in learning and memory task such as eight-arm radial maze task (Ni et al., 1994), Morris water maze task (Ohta et al., 1997). A reduced CBF level further induces oligodendrocytes cell death with DNA fragmentation in which resulted in white matter lesions after 14 days of 2VO onset (Tomimoto et al., 2003). Cerebrovascular white matter lesions caused by CCH is believed to be one of the important pathological factor that is greatly associated with cognitive impairment (Barber et al., 1999). The histopathological changes in white matter is resembling to human leukocephalopathy (Choy et al., 2006). Hence, with all these pathological respects, 2VO in rat is a suitable model to study CCH induced dementia.

2.6 Learning and memory

Cognitive behavior is a series of output response toward external stimuli based on the memory learned from the past experience. Learning and memory are two inter related tasks involved in cognitive process. Learning is the initial stage of memory formation which also known as acquisition stage where new information are acquired (Amin et al., 2014). The newly learned information can be stored in the brain for different length of time through the process of consolidation which moved labile and fragile information into a more solid and fixed state (Abel et al., 2001). Memory is believed to store between the connection of synapse (synaptic plasticity) and through the process of learning the synaptic plasticity is strengthen and leads to generate new synaptic connection. Memory is consider formed when it is able to recall or retrieve, and repetition of this learning and

recall process causes long lasting upregulation of synaptic strength, this process is known as long term potentiation (LTP) (Rioult-Pedotti et al., 2000). The formation of learning and memory appear to be regulated by multiple neurochemical systems including cholinergic, glutamatergic, monoaminergic systems (Myhrer, 2003). Therefore, any interruption occuring in the neurochemical connection leads to several neurodegenerative or neuropsychiatric diseases that results in declining of memory.

2.6.1 Cholinergic system

Numerous studies of cholinergic dysfunction were reported occur in VaD patients and murine models. In bilateral common carotid artery occlusion (BCCAO) of rats has been shown to result in the loss of cholinergic neurons, as demonstrated by decreased choline acetyltransferase and AChE activities (Ni et al., 1995). Rats with 4 vessel and middle artery occlusion (MCAO) occlusions have shown reduction of ACh and impairments in learning and memory (Zhang et al., 2004; Borlongan et al., 2005). The loss of cholinergic neurons originating at the nucleus basalis magnocellularis of basal forebrain and innervates other brain region such as cerebral cortex and hippocampus (Cavedo et al., 2018). Acetylcholine (Ach) is the neurotransmitter that mainly involved the cholinergic signaling activity and often used as a biochemical marker, patient who has dementia usually found to have reduced amount of acetylcholine. Ach is synthesized from acetyl-CoA to choline by enzyme choline acetyltransferase (ChAT). While, there are two types of cholinesterase (ChE): acetylcholinesterase (AChE) and butytrylcholinesterase (BuChE) are responsible for Ach degradation to avoid accumulation of Ach which can lead to over stimulation of muscle and convulsions (Greig et al., 2002). Choline as the precursor of the neurotransmitter acetylcholine can potentially modulate neuronal

plasticity in many ways. First, breakdown of choline into carbon dioxide produces nicotinamide adenine dinucleotide hydrogen (NADH), an electron carrier which can be used by neurons mitochondria to neutralize reactive oxygen species (ROS) in the electron transport chain. Thus, choline plays a vital role in mitigating ROS to maintain neuronal health as neurons have large number of mitochondria in order to supply their high energy demand. Second, betaine which is the first oxidative product of choline, is necessary to produce S-adenosylmethionine, a major group donor for methylation. Studies have shown that methylation through choline supplement can initiate event for the gene activation that involved in cytoskeletal remodeling or synaptic transmission and induce the expression of important cellular signaling protein and transcription factors, such as BDNF, CaMKII, Creb, Gaba.Igf2 and Vegf (Mellot et al., 2004; Blusztajn et al., 2017; Chin and Goh, 2019).

Basal forebrain cholinergic innervates to the hippocampus region are believed to involved in one of the fundamental of learning and memory formation. Sugisaki et al (2011), reported that in high concentration of ACh environment *in vitro*, stimulation of cholinergic neuron that normally generate leng term depression generate long term potentiation (LTP) in hippocampal CA1 region. The underlying mechanism of this effect have clearly elucidated, when ACh binds to muscarinic receptor, causes activation of phospholipase C which is one of the signaling molecule that leads to LTP (Cohen et al., 1998). Furthermore, cholinergic known to be modulate neuroinflammation, the released ACh from the cholinergic neuron is acting on α7 nicotinic acetylcholine receptor (nAChRs) on microglial cells, this activation of microglial cells through nAChRs, decreases inflammatory cytokines (Maurer and Williams., 2017). In addition, Gu et al. (2012) shows

that activation of α7 nAChRs leads to increasing of LTP in hippocampal CA1-CA3 regions, as a result of the long-lasting augmentation of calcium activity.

ACh has been shown to increase BDNF expression via activation of α7 nAChRs and reciprocally α7 nAChRs upregulate expression of BDNF in hippocampus, and thereby increasing synaptic spine formation (Kenny et al., 2000; Massey et al., 2006). Other than that, ACh can also regulate BDNF release via indirect non neuronal pathway, Ruth Barrientos and her collegues, show that inflammatory cytokines-IL-1β which likely released from microglia, modulates BDNF. In her study, mice hippocampal BDNF level is decrease in social isolation stress, and administering IL-1β antagonist to the hippocampus restore BDNF level (Barrientos et al., 2003).

2.7 Synaptic plasticity

Synaptic plasticity is the fundamental for learning and memory, the term "plastic" in synaptic plasticity referred to their characteristic of flexible strength which can be enhanced or weaken, the changes of synaptic strength result in learning and memory. The synaptic strength is dependeds on the mechanism between presynaptic and postsynaptic neurons. According to Hebb's postulation, repeated or persistent firing of presynaptic neuron excites postsynaptic neuron and causes changes in molecular or metabolic at the synapses and eventually synaptic efficiency is strengthen when firing spike of postsynaptic neuron is magnified (Stent, 1973). This upregulation of synaptic strength can sustain for different period of time, when the synaptic strength retain for a few minutes are refer as short-term plasticity. While long-term plasticity can be classified into two phases: early long-term plasticity (e-LTP) and late long-term plasticity (l-LTP) where the former occur at the early phase of LTP that lasts for a few hours and the later involve

synthesis of synaptic protein that occur at the late phase of LTP which last for more than 8 hours to days or even years. Long-term depression (LTD) occurs when firing happens in postsynaptic neuron excites presynaptic neuron (Froemke et al., 2002; Clopath, 2012).

2.7.1 Short term synaptic plasticity (STSP)

STSP can come in several types including paired-pulse facilitation (PPF), post-tetanic potentation (PTP), depression where the synaptic strength lasting from the range of milliseconds (ms) to a few minutes. In mammalian, these mechanisms provide synaptic dynamics for filtering broad range of input information and leads to different postsynaptic firing response depend on the probability of neurotransmitter release (*p*) from the presynaptic (Abbott & Wade, 2004). For instance, if *p* increases reflect facilitation in synaptic, while if *p* decreases reflect depression. These are believed to play a vital role for developing a short-term behavioral changes and simple learning in invertebrate and mammals in order to response to the external stimuli (Citri et al., 2008). The stimulation of short-term synaptic plasticity is initiated by a brief burst of action potential from the presynaptic neuron and elicits a transient elevation of calcium ion in presynaptic neuron causing exocytosis of neurotransmitter from synaptic vesicle to the postsynaptic neuron to evoke potentiation (Zucker & Regehr, 2002; Citri & Malenka, 2008). PPF and PTP are the phenomenon exhibited at the CA3 to CA1 Schaffer collateral pathway of hippocampus and both phenomenons are independent from NMDA receptor.

The experimental evidence of PPF induction is found when two stimuli pulses are delivered simultaneously within a short interval from the range of 20-1000 milliseconds, the evoked potential generated from the second stimulation is commonly larger than the first stimulation (Sweatt, 2010). The postulation of this phenomenon is the calcium residue hypothesis proposed by Katz and Miledi (1968) where the second elicited spike is augmented due to the residual calcium from the first stimulation enhances greater releasing of neurotransmitter from presynaptic neuron and De Camilli and his collegues (1990) have discovered that protein synapsins which tightly modulated by protein kinase in presynaptic has played an important role in neural facilitation. PPF could be involved in processing information but its exact contribution to animal behavior is still not clearly understood.

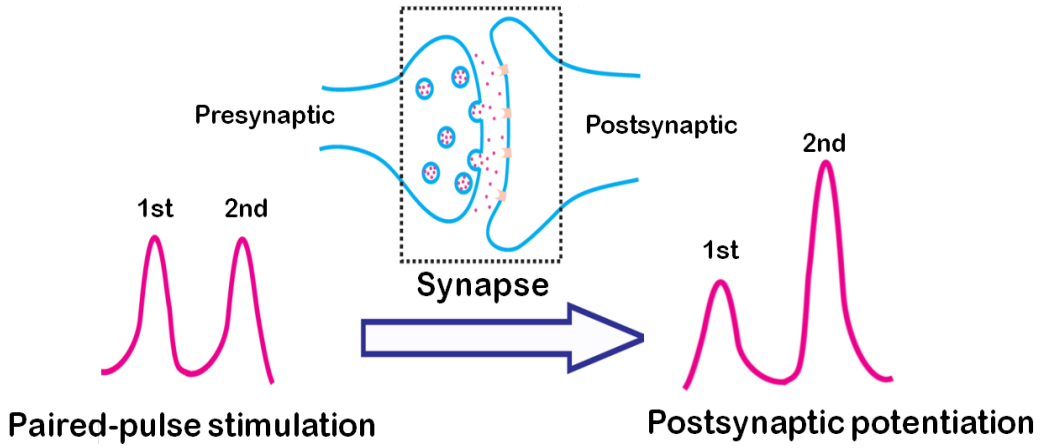

Figure 2.3: Schematic illustration of paired-pulse facilitation (PPF) induction at the presynaptic neuron and the elicited action potentiation at the post-synaptic neuron (Adapted from Hu et al., 2013 with slight modification).

As compared to PPF, PTP introduce greater synaptic enhancement which is induced by higher and repetitive stimulation approximately 10 to 200 Hz for a longer period ranging from 0.2- 5 seconds and the elicited action potential in post synaptic coulds last

for several seconds to minutes (Citri & Malenka, 2008). Evidence has strongly shown that regulation of PTP involved activation of protein kinase C (PKC) and elevation of Ca^{2+} for greater neurotransmitter releases at the glutamatergic synapse, calyx of Held (Balakrishnan et al., 2010). In some cases, repetitive of high frequency stimulation can cause depression instead of facilitation this phenomenon is due to depletion of ready synaptic vesicle pool and it usually found in the synaptic that displayed high initial probability of neurotransmitter such as climbing fiber synapse in PPF activities (Betz, 1970; Abbott & Wade, 2004).

2.7.2 Long-term potentiation (LTP)

LTP is described as an augmented and long-lasting signal for synaptic strengthening between presynaptic and postsynaptic neuron, this mechanism in Schaffer collateral pathway of CA3-CA1 of hippocampus has been a prominent experimental model for studying the mechanism of learning and memory in mammalian at the synaptic level (Bliss & Collingridge, 1993; Arrigoni & Greene, 2004). In LTP experiment, LTP is triggered by theta-burst stimulation (TBS) which made up of ten stimulus bursts delivered at 5Hz, with each burst consisting of five pulses at 100 Hz (Hassan et al., 2019). This pattern of electrical stimulation used in LTP induction, resembles the natural neuronal firing occur in hippocampal CA1 pyramidal neuron in animal and this type of stimulation are discovered with the association of NMDA receptor (Sweatt 2009; Kumar, 2011; Dringenberg et al., 2014).

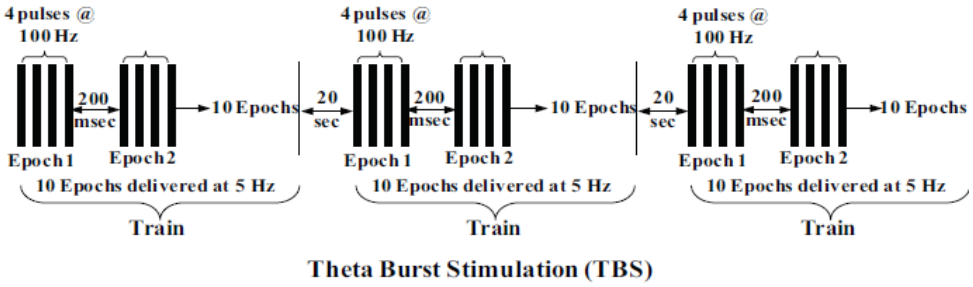

Figure 2.5: Schematic illustration of theta-burst stimulation (TBS) induce LTP (Adapted from Kumar, 2011).

NMDA receptor dependent LTP is discovered by Graham Collingridge in 1983, the first findings discovered the involvement of NMDA receptor in the induction of LTP. In the experiment, amino phosphono valeric acid (APV) used as a blocker for NMDA receptor in rat hippocampal slice and the induction of LTP is failed without interfering the baseline of synaptic transmission (Collingridge et al., 1983).

NMDA is a glutamate gated receptor and acted as a Ca^{2+} channel for depolarizing membrane potential and elicits LTP. The major molecular mechanism involved for LTP induction is well established. Initially, electrical impulses arrived at the presynaptic terminal causing synaptic vesicle to release its neurotransmitter, glutamate into the synaptic cleft. The glutamate binds to AMPA receptors on the membrane of postsynaptic site and causing influx of Na^{+}. When high frequency of stimulation such as TBS is induced, more glutamate are released and causing AMPA to depolarize more Na^{+} and allow the Mg^{+} to repel from hindering the gating of NMDA receptor which has most permeability for Ca^{2+}. As a result, immense influx of Ca^{2+} occurs at the postsynaptic site to cause further depolarization lead to LTP induction by mediate the activation of several protein kinases at different time points (Sweatt, 1999; Kumar, 2011; Morgado-Bernal, 2011).

2.7.2(a) Molecular mechanism of LTP

After TBS, activated protein kinases bind to their specific receptor phosphorylates downstream signaling pathways including coupling of Ca^{2+}/CaMKII (Fukugana et al., 1995), BDNF/TrkB (Minichiello et al., 2002), ERK/MAPK (Bernier et al., 2017), adenyl cyclase/cAMP (Wong et al., 1999) transduction. These activated signaling pathways maximize Ca^{2+} influx and it regulates transcription factors including activation of CREB which is a protein stimulated by cAMP and Elk-1 in the nucleus, causing upregulation of new gene expression for protein required for synaptic structure changes (West et al., 2001).

Ca^{2+} / Calmodulin kinases signaling pathways

CaMKII involved in the process of enhancing and maintaining synaptic transmission in l-LTP. CaMKII contains 28 isomers translate from the genes α, β, γ, δ and the two major subunits comprise are αCaMKII and βCaMKII, (Lisman et al., 2002) which both subunits have different functions in synaptic plasticity (Coultrap & Bayer, 2012). αCaMKII localize abundantly in hippocampus and its activity is require for the formation of memory that involving hippocampus. Yamagata et al., (2009) demonstrated that mice with CaMKIIα activity defect leads poor inhibitory avoidance task performance, but the impairment can be overcome through repetitive training. During the process of LTP, Ca^{2+} binds to calmodulin and form Ca^{2+}/Calmodulin (CaM) complex, followed by autophophorylation of threonine-286 located within the intersubunit of αCaMKII, while βCaMkII subunits binds to F-actin. The phosphorylation of αCaMKII leads to dissociation of whole unit of CaMKII from F-actin. Detach of CaMkIIβ and the free F-actin involved the cytoskeleton reconstruction that cause structural changes in dendritic spine (postsynaptic site), such as enlargement of dendritic spine, which is crucial in the l-LTP

for synaptic strength maintenance (Okamoto et al., 2009; Morgado-Bernal, 2011; Khan et al., 2016).

On the other hand, activity of phosphrylated CaMKII is lengthen due to the autophosphorylation at threonine-286 of CaMKIIα, activated CaMKII binds to the stargazin part of AMPA receptor which allow AMPA receptor migrate to the protein dense specialization known as postynaptic density (PSD) on the postynaptic membrane (Giese & Mizuno, 2013; Lisman et al., 2014). The simple illustration of the CaMKII mechanism is shown in Figure 2.5. In addition, the source of AMPA receptor inserted to the membrane of postsynaptic neuron is supply continuously from endocytosis of vesicle containing AMPA receptor (recycling endosome) upon LTP induction (Park et al., 2004). Therefore, synaptic transmission is enhanced due to the increases of AMPA receptor insertion into the PSD and AMPA receptor associated with the persistent CaMKIIα activation leads to prolong activity of AMPA regulated synaptic transmission such as LTP (Yamagata et al., 2009).