NEUROPROTECTIVE EFFECTS OF EDIBLE BIRD'S NEST IN CHRONIC CEREBRAL HYPOPERFUSION-INDUCED NEURODEGENERATION IN RATS

BY

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ABSTRACT

Vascular dementia (VD) is the second most prevalent type of dementia after Alzheimer's disease (AD) dementia. It is marked by insufficient blood supply to the brain that leads to progressive loss of memory and cognitive skills. Continuous reduction in cerebral blood flow, which made by chronic cerebral hypoperfusion, leads to overproduction of reactive oxygen species together with reduction in the brain glucose and oxygen that causes cognitive decline. Until now there is no available curative treatment for VD and the only available option is symptomatic treatment. Several studies have shown that recent alternative medicines have underscored the neuroprotective and antioxidant ability of the edible bird's nest (EBN). Nevertheless, there has been minimum studies explored the effect of EBN in reducing the risk of VD. The current study evaluates the effects of EBN on hippocampal neurons mainly in CA1 hippocampal region in chronic cerebral hypoperfusion rat model. Chronic cerebral hypoperfusion model was created by permanent occlusion of bilateral common carotid arteries (2VO) in rats that induced inflammation in brain neuronal cells. The rats were divided into 4 groups: A) Sham group, B) 2VO group, and Groups C and D are 2VO rats treated with (60,120 mg/kg) of oral EBN. After 8 consecutive weeks, the rats were killed and the hippocampi were examined histopathologically by counting the viable neuronal cells in hippocampus CA1 area and the level of F2 Isoprostane, an oxidative stress indicator, was measured. The results showed a significant reduction in the neuronal cell death and significant decline in F2 Isoprostane level in the group of rats treated with EBN when compared to untreated 2VO. This is the first study that links the possible neuroprotective effects of EBN in in chronic cerebral hypoperfusion. The results might have a great impact in the application of EBN in delaying the progression of dementia in AD patients.

خلاصة البحث

يعد مرض الزهايمر اكثر انواع الخرف الوعائي ويتميز بعدم كفاية امداد الدم الي الدماغ مما يؤدي إلى فقدان الذاكرة والمهارات المعرفية بشكل تدريجي. استمرار انخفاض تدفق الدم الدماغي، والذي يحدث بسبب نقص تدفق الدم الدماغي المزمن، يؤدي إلى زيادة إنتاج أنواع الأكسجين التفاعلية مع انخفاض الجلوكوزوالأكسجين الذي يتسبب في تدهورالإدراك و حتى الآن لا يوجد علاج علاجي متاح لـمرض الزهايمر والخيار الوحيد المتاح هو علاج الأعراض. أظهرت العديد من الدراسات أن الأدوية البديلة الحديثة قد أكدت على قدرة عش الطيور الصالحة للأكل (EBN)على الحماية العصبية ومضادات الأكسدة. ومع ذلك ، كان هناك الحد الأدنى من الدراسات التي استكشفت تأثير EBN في تقليل مخاطر VD. تقيّم الدراسة الحالية تأثيرات EBN على الخلايا العصبية في الهبوكمبس بشكل رئيسي في منطقة CA1 في نموذج الفئران الناجم عن نقص تدفق الدم الدماغي المزمن. تم إنشاء نموذج نقص انسياب الدم الدماغي المزمن عن طريق الانسداد الدائم للشرايين السباتية الثنائية المشتركة (VO2) في الجرذان التي تسبب الالتهاب في الخلايا العصبية. تم تقسيم الجرذان إلى 4 مجموعات: أ) مجموعة الكنتورل ، ب) VO2 المجموعة ، والمجموعات C و D عبارة عن جرذان VO2 تعامل بجرعتين مختلفتين من EBN الفموي. بعد 8 أسابيع ، قُتلت الفئران وفُحص الهبوكمبس من الناحية النسيجية عن طريق عد الخلايا العصبية القابلة للحياة وقياس مستوى F2 Isoprostane ، وهو مؤشر الإجهاد التأكسدي. أظهرت النتائج انخفاضًا كبيرًا في موت الخلايا العصبية وانخفاض معنوي في F2 Isoprostane في مجموعة الفئران التي عولجت بـ EBN. هذه هي الدراسة الأولى التي تربط التأثيرات الوقائية العصبية لـ EBN في الوقاية من الخرف المرتبط بمرض الزهايمر. سيكون للنتائج تأثير كبير في تأخير تطور الخرف لدى مرضى الزهايمر

APPROVAL PAGE

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AD	Alzheimer’s disease
APP	Amyloid precursor protein
ATP	Adenosine triphosphate
AA	Archidionic Acid
AβO	Aβ oligomers
BBB	Blood brain barrier
BCCAO	Bilateral common carotid artery occlusion
CAT	Catalase
CA-1	Cornu ammonis
CCH	Chronic cerebral hypoperfusion
CBF	Cerebral blood flow
COX	Cyclooxygenase
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
ELISA	Enzyme linked imunosorbent assay
FTD	fronttemporal dementia
GSpx	Glutathione peroxidase
H&E	Hematoxylin and eosin
HD	Hunington’s disease
F2-IsoPs	Isoprostanes F2
MLS	Amyotrophic lateral sclerosis
MtD	Mitochondrial dysfunction

MAD	Malondialdehyde
MCI	Mild cognitive impairment
MWM	Morris water maze
NFTs	Neurofibrillary tangles
NSAIDS	Non-steroidal anti-inflammatory diseases
OS	Oxidative stress
PUFA	Polyunsaturated fatty acids
PG	Prostaglandin
P Tau	Phosphorylated tau
PSEN1	Presenilin 1
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
SOD	Superoxide dismutase
2VO	2 Vessel occlusion
Vit E	Vitamin E
2VO+E	2 Vessel occlusion + Vitamin E
VD	Vascular Dementia
WM	White matter

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Neurodegenerative diseases are marked by gradual loss of neurons and synapse in central nervous system, which leads to loss of functions and death of neuronal cells. Although therapeutic treatments may help in relieving some of the associated symptoms, there is no available curative treatment to slow down the progression of the disease (Kovacs, 2016, 2018; Walsh & Selkoe, 2016). Alzheimer's disease (AD) is the most serious cause of mental deterioration and neurological disorder that leads to loss independency of life in elderly people. It is a chronic disease associated with excessive loss of neurons particularly in the hippocampus and cortical region that leads to cognitive impairment (Querfurth & LaFerla, 2010; Wang et al., 2020). The key pathological features of AD are the accumulation of amyloid-β (Aβ) in extracellular forming plaques as result of the cleavage of amyloid precursor protein (APP) (de Strooper & Karran, 2016; Palop & Mucke, 2010; Selkoe & Hardy, 2016; Sun et al., 2018a) along with formation of intracellular neurofibrillary tangles that consist of tau protein (Bejanin et al., 2017a 2017c; de Calignon et al., 2012; Sepulcre et al., 2016). Until now the etiology of AD remains uncertain. In familial Alzheimer's disease (FAD), it is hypothesized that most of cases are caused by mutation in amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2). Other cases are Sporadic Alzheimer's Disease (SAD), which is a late-onset form of AD due to different risk factors including genetic and environmental exposure (Bendlin et al., 2010; Gatz et al., 2006). Aging is the biggest risk factor for AD where,

the clinical symptoms of AD are usually developed at age older than 65 year (late-onset AD) (Balin & Hudson, 2014; Pierce et al., 2017). However, 2% to 10 % of patients have early onset familial AD form in the middle age that causes minor memory loss but progresses to major cognitive dysfunction, motor difficulties and severe memory loss (Mendez, 2017, 2019; Wattmo & Wallin, 2017). The number of people suffering from AD related dementia is massively increased worldwide. There are more than 47 million people suffering from dementia all over the world, which is predicted to be doubled in every 20 years, increasing to more than 131 million by the year 2050 (Kongburan et al., 2019; Prince et al., 2013).

Oxidative stress and inflammation have been considered to be the main causes in the etiology of AD that are directly damaging the neurons (Du et al., 2018; Liu & Zhang, 2012). Chronic cerebral hypoperfusion (CCH) along with various types of vascular insufficiency may initiate a cascade of incidences such as, oxidative stress and inflammatory reactions that attributed to initiation of vascular dementia (Liu & Zhang, 2012; Navarro et al., 2018; Sarlus & Heneka, 2017). As a proven animal model for imitating the decrease in cerebral blood flow in human aging. Permanent bilateral occlusion of common carotid arteries of rats (2-Vessel Occlusion, 2VO) has been introduced (Farkas et al., 2007a). A sustained reduction in regional cerebral blood flow (CBF) in different brain areas including cerebral cortex and hippocampus causes neuronal dysfunction and impairment in spatial learning leading to memory deficits and dementia (de la Torre, 2018; Duncombe et al., 2017). Previous studies of neuropathological changes caused by CCH focused on the hippocampus considering its critical role in learning and memory (Du et al., 2013; Patel et al., 2017; Pirmoradi et al., 2019). Furthermore, the hippocampal CA1 region is the most vulnerable area to

the effect of CCH (Navarro et al., 2018; Saxena et al., 2015; Wang et al., 2020). Several studies have further discovered that there is a relation between cognitive functions and the numbers of healthy neurons in the hippocampal CA1 area. Therefore, neuroprotection can lead to positive outcomes in preserving cognitive abilities. (Pirmoradi et al., 2019; Wang et al., 2020; Wang et al., 2020). Neuroprotection can be achieved via several natural products as adjuncts to pharmacological treatment in AD such as Resveratrol, andrographolide, Vitamin E, and edible bird's nest (EBN) (Baghcheghi et al., 2020; Wang et al., 2020; Wang et al., 2019).

EBN is one of the world's most valuable animal products eaten by humans, either for its medicinal properties or as a unique tasty food in Southeast Asia (Ma & Liu., 2012; Chua et al., 2014). It is made from the saliva of swiftlets birds during breeding and nesting season. The market price ranges from $1000 to $10000 per kilogram according to the quality and type of EBN. Researchers investigated the therapeutic effects of EBN such as antioxidant, anti-inflammatory, influenza virus inhibitory effect, bone strengthening, and neuroprotection (Albishtue et al., 2018; Haghani et al., 2017a; Yida, Imam, Ismail, Ismail, et al., 2015). EBN is a great source of protein, carbohydrate, amino acids and some trace elements and also has contains epidermal growth factor like activity (EGF) (Kong et al., 1987; Marrocco., 2005). Apart from that, it also contains lactoferrin and ovotransferrin type of glycoproteins that interact with free radical species inhibiting oxidative stress reactions in hydrogen peroxide induced oxidative stress in human SH-SY5Y cell (Hou et al., 2015). It has been reported to exhibit neuroprotective role in human neuroblastoma cell model through attenuation of oxidative stress (Yew et al., 2014). Sialic acid is one of the

most important constituents in EBN (Careena et al., 2018; Pozsgay et al., 1987). It is an essential nutrient to the brain in terms of neuronal outgrowth, synaptic connectivity and memory formation. It was also shown that eating diet rich in sialic acid enhances learning and memory (Bing Wang, 2009, 2012).

In order to diagnose and treat the pathophysiological changes in CCH, the deduction of essential biomarker could have a critical function (Marrocco et al., 2017). F2 isoprostanes (IsoPs) are prostaglandin-like molecules formed from the peroxidation of arachidonic acid. F2-IsoPs are considered accurate indicators for lipid peroxidation oxidative stress due to their stability in biological samples (Arikawa et al., 2017; Roberts & Milne, 2009). The accumulation of F2-IsoPs in the cerebrospinal fluid (CSF) in brain has an important etiological implication for neurodegenerative disorders such as AD (Montine et al., 2011). It was found that the determination of IsoPs level in CSF might boost laboratory diagnostic efficacy for AD while, assessment of IsoPs in urine and plasma of AD patient had shown conflicting results (Montine et al., 2004a; Montine et al., 1998; Montine et al., 2002, 2005, 2011).

1.2 PROBLEM STATEMENT AND JUSTIFICATION OF THE STUDY

AD is the most prevalent neurodegenerative disorder that causing dementia, which has been developed rapidly in last 60 years. All studies strongly support that AD is a chronic disorder that starts years before clinically cognitive damage is detectable. The challenges in AD research today include the strategies for early identification of patient and discovering new therapies for disease prevention and cure. Tens of millions of people all over the world is suffering from AD that expected to be increased to over 130 million in 2050 (Prince et al., 2013; Alzheimer Disease International., 2015). The incidence of dementia related to AD in Malaysia in the 2020

and 2050 is predicted to be 0.126% and 0.454% respectively (Cazarim et al., 2016; Tey et al., 2016). AD is considered one of the biggest socio-economic problems that cost billions of dollars in medical care worldwide especially with disease progression (Castro et al., 2010; Cazarim et al., 2016). It has expanded worldwide in spite of the improvement in the field of drug discovery due to its associated long-term complications. Hence, there is a demand for new drugs that may modify the progression of the disease and its complications. However, there were no drugs available to fight against the pathophysiology of AD (Cazarim et al., 2016). Currently the only therapies available for AD are to improve the symptoms of disease but not disease-modifying agents that may treat the original neurodegenerative process (Fanoudi et al., 2019; Luca et al., 2015).

Several studies showed that reactive oxygen species (ROS) are essential factors in AD pathogenesis (Fanoudi et al., 2019; Pirmoradi et al., 2019; Yao et al., 2019). While the lack of ROS protection in aging brain leads to progression of the disease. Consequently, using natural antioxidants to prevent and treat AD is a mandatory for disease management (Zhang et al., 2018; Nalivaeva & Turner, 2016). EBN is a promising natural food that has antioxidant and anti-inflammatory effect (Yew et al., 2014; Yida, Imam, Ismail, Ismail, et al., 2015; Zhiping et al., 2015). It contains higher bioactive compounds such as Sialic acid, glycoprotein and minerals, which helps to improve metabolism and physiological functions of the neurological systems with a neuroprotective effect that enhances memory (Aswir & Wan Nazaimoon, 2011; Hou et al., 2017a; Wong et al., 2018; Xie et al., 2018). The current study investigates the neuroprotective effect of EBN using chronic cerebral hypo perfusion- induced neurodegeneration in rats.

1.3 GENERAL OBJECTIVE

To assess the neuroprotective effect of EBN on neuronal cell survival and oxidant level in hippocampus of 2VO rat model.

1.4 SPECIFIC OBJECTIVES

1. To compare the viable neuronal cells in the hippocampus between EBN treated groups and untreated groups of 2VO rat model induced neurodegeneration.
2. To compare F2 Isoprostane level in the hippocampus tissue between treated groups with EBN and untreated groups of 2VO rat model.

1.5 RESEARCH QUESTION

Does EBN has neuroprotective effects in 2VO rat model of neurodegeneration?

1.6 RESEARCH HYPOTHESIS

EBN has neuroprotective effects against 2VO rat model of neurodegeneration.

CHAPTER TWO

LITERATURE REVIEW

2.1 NEURODEGENERATIVE DISORDERS

Neurodegenerative disorders (NDs) are the most prevalent diseases worldwide. The common features of NDs are the aggregation and misfolding of specific proteins (Kovacs, 2016, 2018; Walsh & Selkoe, 2016) that lead to cellular abnormalities, loss of synaptic connection, changes in physicochemical properties in brain and in peripheral organs cause disease progression (Alzheimer's Association, 2020; Querfurth & LaFerla, 2010; Soto& Pritzkow, 2018). The classification of NDs is mainly based on the clinical symptoms and biochemical modification due to protein aggregation in the intercellular or extracellular spaces such as Alzheimer's disease (AD), frontotemporal dementia (FTD), Parkinson's disease (PD), Multiple sclerosis (MA), Amyotrophic lateral sclerosis (MLS) and Huntington's disease (HD) (Kovacs, 2016, 2018).

2.2 ALZHEIMER'S DISEASE (AD)

In year 1907, Professor Alois Alzheimer, a psychiatrist with expertise in neuropathology was the first one identified AD. AD is a chronic irreversible disease, which is identified by insidious and cumulative loss of neurons particularly in the cerebral cortex and hippocampus. The main pathological hallmarks of AD are dementia and cognitive impairment. Definite diagnosis of AD can only be made postmortem where neurofibrillary tangles and amyloid plaques are the two main characteristic clinical features (Busche et al., 2019; Cline et al., 2018; Tu et al., 2014). Clinically, AD patients usually have demented memory, learning disability and

behavioral disturbances, which are associated with language and depressive symptoms that contributed to the decline in all mental activities and finally death. Aging is the main contributing factor for the rising of AD prevalence worldwide. At age of 70-years old the early stage of AD starts where neurons degenerate leads to mild cognitive dysfunction then increased by time to sever cognitive deterioration. The progression of disease triggered by several vascular risk factors such as diabetes type-2, hypertension, dyslipidemia, smoking, obesity, or atherosclerosis (Chornenkyy et al., 2019; Akinyemi et al., 2013; Silva et al., 2019).

According to the World Health Organization (WHO), AD is the fifth global leading cause of death. Recently, the growing death rate from this disorder has become significant with an expected rise of over 130 million by 2050 (Eggink et al., 2019; World health organization, 2018). AD is a great socio-economic problem, not only patients will suffer but also a burden to their relatives and caregivers. The health care cost of the diagnosis, prevention and treatment is a burden on the economy aspect. In-addition to the time used to treat the patient increased with the progression of the disease. Also, current therapeutic strategies provide only a palliative treatment not curative one (Castro et al., 2010; Cazarim et al., 2016). AD is not only a research issue, but it has also psychological and ethical impacts together with damaging effect on economy.

The consequences of anatomical change in the brain occur as aggregation of β amyloid peptides and tau protein cause synapses failure and deterioration in various regions of the brain (Busche et al., 2019; Cline et al., 2018; Shu et al., 2018; Tu et al., 2014). Family history, physical and laboratory examination are used in the diagnosis and assessment of AD. In addition to neuroimaging that is helpful in distinguishing AD from other neurological disease (Rathore et al., 2017; Sørensen et

al., 2017). Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) are the only ways to detect the advanced stage of AD (Moretti, 2015). So, using other biomarkers in blood, plasma and urine helps in early diagnosis of AD. Early diagnosis promotes not only our understanding of the neurological complication but also helps us in handling and early treatment of these patients.

While the etiology of AD is not clearly understood, the inheritance of genetic factors plays a major role after age in the AD pathogenesis (Tanzi, 2012). Early onset of FAD in middle age occurs in about 5% to 6 % of all diagnosed AD patients. It has more aggressive course that starts with minor memory loss then progresses later to major cognitive dysfunction with severe memory loss and motor difficulties (Mendez, 2017, 2019; Wattmo & Wallin, 2017). Mutation in APP, PSEN1 and PSEN2 are assumed to be associated with the early onset of etiology (Lanoiselée et al., 2017). On the other hand, late-onset AD account for over 95 % of all AD cases. The prevalence and incidence of AD are rapidly increasing after age of 65 and doubled in every 5 years (Balin & Hudson, 2014; Pierce et al., 2017). Neuronal dysfunction is critically affected by depositions of β amyloid peptide and neurofibrillary tangles (NFT) in both early- and late-onset AD (Bejanin et al., 2017c, 2017a; Busche et al., 2019; Sepulcre et al., 2016; Sun et al., 2018b). Insufficient cerebral blood flow also contributes to the progression of dementia (de la Torre, 2018; de La Torre, 2004, 2010, 2012, 2013; de la Torre, 2002a; de la Torre, 2010; Farkas et al., 2005, 2007a; Farkas & Luiten, 2001; J.C., 2010; Royall & de la Torre, 2002; Wang et al., 2020; Won et al., 2013). Continuous microglial activation and higher rates of proinflammatory cytokines characterize the brain aging and neurodegeneration along with increased in oxidative stress and neuronal damage (Nalivaeva & Turner, 2016; Sarlus & Heneka, 2017). The main histopathological hallmarks of AD are the senile plaques composed of

extracellular Aβ and intraneuronal NFTs of Tau protein (Bejanin et al., 2017b; Morrone et al., 2020; Selkoe & Hardy, 2016; Sun et al., 2018a; Takahashi et al., 2017) The irreversible symptoms of AD were observed when both NFTs and beta Aβ are found in limbic system.

2.3 AD HYPOTHESES AND PATHOGENESIS

The pathogenesis of AD still remains uncertain with several postulated hypotheses, which include mainly amyloid cascade, vascular, oxidative stress, and inflammation processes.

2.3.1 The Amyloid Cascade Hypothesis

The amyloid hypothesis has become the dominant theory of AD pathogenesis and has contributed to potential therapeutic advancement (Cline et al., 2018; Selkoe & Hardy, 2016; Takahashi et al., 2017). Aβ42 was believed to be the preliminary factor of early phase of AD due to the imbalance between their production and clearance. It started many years before the onset of AD symptoms. Mutations in presenilin 1 or 2 and the amyloid precursor protein (APP) are the most frequent cause of early-onset AD. These mutations lead to higher generation of Aβ42/43 peptides with decreased in its clearance resulting in excessive Aβ42 aggregation that forming oligomerization. Increased intercellular Ca^{+2} and oxidative stress were triggered by Aβ oligomers (AβO) in addition to activation of microglial and astrocytes that lead to functional impairment of normal cellular lipids, proteins, and DNA as well as decrease synapse density (Alberdi et al., 2010; Busche et al., 2019; de Strooper & Karran, 2016; Palop & Mucke, 2010; Takahashi et al., 2017). Furthermore, it is associated with the aggregation and induction of phosphorylated microtubule-associated protein tau (P-