UNIVERSITI TEKNOLOGI MARA

NEUROPROTECTIVE AND ANTINEUROINFLAMMATORY EFFECTS OF *MYRMECODIA PLATYTYREA* TUBER AQUEOUS EXTRACT

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ABSTRACT

Myrmecodia platytyrea (Family: Rubiaceae) is commonly known as the Ant-nest plant and locally as *Sarang Semut*. It is used in Indonesian traditional medicine for the management of oxidative stress- and inflammation-related diseases such as cancer, diabetes mellitus, cardiovascular diseases and could have potential in treating Alzheimer's disease (AD). Hence, our study aimed to investigate the potential of *M. platytyrea* tuber aqueous extract (MPAE) in inhibiting neuroinflammation, *in vitro* and *in vivo*. The *in vitro* antineuroinflammatory effects of MPAE (0.025 - 0.5 mg/ml) were investigated by measuring the cytotoxicity and proinflammatory cytokines (TNF-α, IL-1β and IL-6) production in $FeSO_4$-, H_2O_2- and LPS-stimulated astrocyte cell line. For the *in vivo* study, MPAE was assessed in non-LPS mice and LPS-neuroinflammation mice model by Morris water maze (MWM) test. ICR male mice aged 24 weeks were grouped into four and six groups (n=6/group), respectively to each model. For first model, Group 1 was pretreated with distilled water (10 ml/kg, p.o.) while Group 2-4 with MPAE (100, 200 and 400 mg/kg, p.o.). In LPS-model, Group 1 and 2 were pretreated for six days with distilled water, Group 3 with standard nootropic agent, piracetam (400 mg/kg, p.o.) and Group 4-6 with MPAE (100, 200 and 400 mg/kg, p.o.). Then, LPS (3 mg/kg; i.p) was administered to the mice of Group 2-6 for 3 days. The mice were subjected to 2 days of training followed by 3 days of MWM test and a day of probe test. Next, the brain was collected for bioassay analysis and molecular works. The antioxidant enzymes (SOD, CAT, GPx), inflammatory cytokines (TNF-α, IL-6, IL-1β), cholinergic activities (ACh and AChE) and amyloid protein ($A\beta_{1-40}$ and $A\beta_{1-42}$) assays were conducted using ELISA assay. Meanwhile, inflammatory markers (COX-1, COX-2, PGE_2, iNOS and NF$\kappa\beta$) were determined via RT-PCR from the brain homogenate. From the results, MPAE was not cytotoxic on astrocytes with IC_{50} value of 1.54±0.26 mg/mL. However, MPAE did not protect the astrocytes against Fe_2SO_4, H_2O_2, or LPS but demonstrated an increment of cell death in the astrocytes in a dose-dependent manner. The level of the cytokines was increased dose-dependently. Treatment of MPAE on non-LPS mice significantly ($p<0.05$) worsened the memory and learning, decreased the antioxidant activity and increased the inflammatory cytokines, inflammatory mediators, cholinergic activities and production of Aβ protein compared to control mice. In LPS-neuroinflammation mice model, administration of LPS caused a significant ($p<0.05$) cognitive impairment compared to control mice. Remarkably, mice that received piracetam and MPAE (200 mg/kg) were significantly ($p<0.05$) improved the memory and learning in MWM test. MPAE (200 mg/kg) was significantly ($p<0.05$) increased the antioxidant enzyme activities compared to LPS group. MPAE (200 mg/kg) was significantly ($p<0.05$) reduced the proinflammatory cytokines and inflammatory mediators compared to LPS group. Consistently, MPAE (200 mg/kg) significantly ($p<0.05$) inhibited the production of Aβ peptides and elevated concentration of ACh while inhibited AChE compared to LPS group. To conclude, MPAE has a potent neuroprotective agent and antineuroinflammatory effect via modulation of the inflammatory mediators in LPS-induced neuroinflammation. Thus, suggesting MPAE having potential therapeutic application against neuroinflammation.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Neuroinflammation is a brain's activation of the innate immune system functions to protect the central nervous system (CNS) against infectious insults, injury, or disease (Zhang and Jiang, 2015). It has been well established that neuroinflammation is actively involved in neurological diseases and disorders such as Alzheimer's disease (AD) (McGeer and McGeer, 2010). It is a complex response involving a host of cellular and molecular changes, recruitment of peripheral immune cells, induction of some intracellular signalling pathways, and release of inflammatory mediators in the brain. All these factors can contribute to the occurrence of neuronal dysfunction and death in AD, either alone or in combination. These observations and other researches indicate that neuroinflammation is an early and continuous feature of AD (Morales et al., 2014).

AD is a neurodegenerative disease and is the most common form of dementia and accounts for more than 80% of dementia cases worldwide in elderly people (Anand et al., 2014; Ulep et al., 2017). It leads to the progressive loss of mental, behavioural, functional decline and ability to learn (Anand et al., 2014). The neuropathological features of the disease are the aggregation of Aβ and hyperphosphorylation of tau protein (Castellani, et al., 2014). Five drugs have been approved by the U.S. Food and Drug Administration (FDA) viz. cholinesterase inhibitors (donepezil, galamantine, rivastigmine and tacrine) and *N*-Methyl-D-Aspartate (NMDA)-receptor antagonist (memantine) which provide modest benefits to treat the cognitive symptoms of AD (Casey et al., 2010). At present, there is no treatment that can reverse, stabilize or even delay the course of this progressive dementing disorder (Honig & Boyd, 2013). A variety of side effects are observed with these drugs which are cholinergic in nature and include effects on the brainstem (nightmares); on the neuromuscular junction (leg cramps); vagal heart innervation (bradycardia or syncope) (Honig & Boyd, 2013). Since AD is irreversible, there is a need to uncover and develop new neuroprotective agents to halt development and progression of the disease.