UNIVERSITI TEKNOLOGI MARA

LACTOBACILLUS PLANTARUM LAB12- AND *LACTOBACILLUS CASEI* LAB13- INDUCED NEUROPROTECTION WAS ASSOCIATED, AT LEAST IN PART, WITH INCREASED NEUROTRANSMISSION, ANTI-OXIDANT AND DECREASED NEUROINFLAMMATION IN APP TRANSGENIC MICE

NOR AMALINA BT AHMAD ALWI

Thesis submitted in fulfillment
of the requirements for the degree of
Master of Science

Faculty of Pharmacy

April 2018

ABSTRACT

Bidirectional signalling between the gut and the brain plays essential role in maintaining homeostasis of our body. This interesting concept of gut-brain axis has led to discovery of gut microbiota-modulated neuroprotection. The mechanisms underlying this newly uncovered therapeutic effect remain poorly understood. Alzheimer's disease (AD) is a neurodegenerative disorder that poses vicious threats to health and well-being of the ageing world population. It is characterised by increased oxidative stress and neuroinflammation, as well as reduced neurotransmission. Currently, there is increasing evidence regarding the effective use of probiotics or beneficial bacteria as neuroprotective agents against AD. The present study had successfully elucidated mechanisms underpinning neuroprotection induced by the unique *Lactobacillus plantarum* (LAB12) and the commercialized *Lactobacillus casei* strain Shirota (LAB13) using APP transgenic mouse model. The mice (male; 10-12 months old) were treated with LAB12 (n = 6) and LAB 13 (n = 6), respectively (10^9 CFU/mL, p.o) over a period of 12 weeks before being subjected to behavioural study using the Morris Water Maze Test. The transgenic rodents were then sacrificed and brains were removed. Brain homogenates were subjected to amyloidogenic genes expression, cholinergic, antioxidative and anti-inflammatory tests. Results from the behavioural study indicated enhanced memory and learning abilities in LAB-supplemented groups. LAB-supplemented transgenic mice yielded reduced escape latency and distance over three consecutive days when compared to their APP control and wild type counterparts. The time spent by LAB 12- and LAB 13-supplemented mice in platform zone during Probe Test was significantly ($p < 0.05$) increased by 57% and 66%, respectively. In term of the amyloidogenic pathway, LAB12 and LAB13 inhibited BACE1 (+28% vs +20%) and APP (-42% vs -44%), both of which are contributors to Aβ production. Unlike LAB12 which had exhibited no significant effect on the cholinergic pathway, LAB13 significantly increased the neurotransmitter, ACh (+23%; $p < 0.01$), and significantly reduced AChE (-13%; $p < 0.05$) that degrades ACh. Whilst LAB13 increased CAT (+33%; $p < 0.01$) and GPx (+43%; $p < 0.05$), LAB12 increased CAT (+27%; $p < 0.05$) and GSH (+40%; $p < 0.05$), all of which are excellent antioxidant enzymes that protect against oxidative stress (i.e. NO). The up-regulated antioxidant enzymes might have suppressed oxidative stress in the brain given the nitric oxide (NO) level in LAB12- and LAB13-supplemented groups that was significantly ($p < 0.05$) reduced by 50% and 75%, respectively when compared to control. Whilst there were modest changes amongst the tested pro-inflammatory cytokines (IFN-γ, IL-1β and IL-6), the anti-inflammatory IL-10 was significantly ($p < 0.01$) up-regulated in LAB-supplemented groups as opposed to control. IL-10 was found to be increased by 21% and 25% in transgenic mice fed with LAB12 and LAB13, respectively. Altogether, the LAB-induced neuroprotection could be mediated, at least in part, through regulation of cholinergic activity, anti-oxidative activity and anti-inflammatory activity. The present findings provide important insights into design of therapy that can further enhance LAB-induced neuroprotection in AD.

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful, all praises and thanks be to Allah for His blessings. For without His guidance and mercy, I would not have been able to complete this dissertation.

I would like to humbly express my gratitude to all who had contributed, either directly or indirectly, towards the successful completion of my postgraduate journey. First and foremost, I would like to express my sincere appreciation to my supervisor, Dr. Lim Siong Meng and co-supervisor, Associate Prof. Dr. Kalavathy Ramasamy. This work would not have been possible without their supervision, guidance, encouragement and endless support. I thank them for all their advice and motivation from the beginning till the end of my candidature. Special thanks to Associate Prof. Dr Vasudevan Mani for his guidance on the part of my project which required animal handling.

I am truly grateful for the financial support by the Ministry of Science, Technology and Innovation (MOSTI), and Ministry of Higher Education (MOHE). I acknowledge receipt of funding under the e-Science Grant [100-RMI/SF 16/6/2 (10/2012)] and the Long Term Research Grant Scheme [600-RMI/LRGS 5/3 (2/2012)].

My heartfelt thanks go to all members of the Collaborative Drug Discovery Research (CDDR) Group: Mdm Azidah Ali, Mdm Nurul Huda Musa, Mdm Nor Nadia Ban, Mdm Hanum Yaakub, Ms Nur Syafiqah Rahim, Ms Siti Aisyah Sayadi, Ms Che Adlia Che Ady, Mdm Dayana Sazereen Md Hasni, Mr Muhammad Zaki Ramli, Mr Muhamad Fareez Ismail, Mdm Syamimi Samah and Mr Mohd Zaki Zakaria; the Brain Degenerative and Therapeutics Group: Mdm Siti Munirah Jaafar and Mdm Nur Syamimi Mohd Azahan, and fellow friend from the Universiti Teknologi MARA (UiTM), Mdm Maisarah Mohd Zin, for all their assistance, kindness, cooperation and moral support. I am beyond grateful for our friendship and wonderful memories over the course of my journey.

Last but not least, I wish to dedicate my greatest appreciation to my beloved parents, Mr Ahmad Alwi Abd Rashid and ; my siblings (Nor Hayati, Ahmad Shahrul, Noor Huda, Ahmad Musthofa and Nur Syakirah) and other family members. No words can utter how much I cherish their endless love, prayers and encouragement through the many ups and downs. To those whose names are not mentioned here but had contributed to this research in one way or the other, your kindness meant a lot to me.

I am very proud to have completed yet another remarkable achievement in my life. I thank all of you from the bottom of my heart. There is no way I can possibly repay all your good deeds. It is my prayer to Allah that He will shower His mercy and blessings upon all of you.

TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

The global elderly population is on the rise given the improved living standard and reduced rates of mortality and fertility. Number of people aged 60 years and above are expected to escalate by 1.25 billion come 2050 (Prince et al., 2013), increasing the susceptibility to chronic and degenerative diseases like Alzheimer's disease (AD). AD, a neurological disorder characterised by progressive loss of memory and cognitive function, causes loss of independence (Bateman et al., 2012). It is the commonest form of dementia that affects individuals over 65 years of age (Ballard et al., 2011; Brookmeyer et al., 2007). The prevalence of AD is estimated to reach 106.2 million by 2050 (Norton et al., 2014). Whilst deaths associated with AD had increased by 68% between 2000 and 2010, deaths attributed to other major diseases (e.g. heart disease) had decreased over the same period of time (Association, 2013a, 2013b). In Malaysia, it is estimated that 50,000 people are currently affected by AD. The number, however, could be higher given that not all who suffer from AD are officially diagnosed (ADFM, 2014).

Accumulation of intracellular neurofibrillary tangles (NFT) and extracellular senile plaques in the brain are the two main hallmarks of AD (Tillement et al., 2011). Whilst NFT are aggregates of microtubule-associated tau protein, senile plaques are predominantly comprised of amyloid-β (Aβ) deposits. Even though the exact etiology of AD remains to be fully elucidated, findings from genetic, biochemical, and *in vivo* studies appear to suggest the potential role of Aβ as the main causative factor in pathogenesis of AD (Wildsmith et al., 2013). Basically, Aβ is derived from sequential proteolytic cleavage of amyloid precursor protein (APP) by β-APP cleaving enzyme (BACE1) followed by γ-secretases (Ahmed et al., 2010). Aβ peptide at low doses plays essential roles at the synapse. It produces presynaptic enhancement, increases neurite outgrowth and is involved in regulation of cholinergic neurotransmission (Morley & Farr, 2014; Sadigh-Eteghad et al., 2015). Subsequent aggregation of Aβ, however, could mediate toxicity that impairs synaptic function, which could in turn lead to progressive memory loss and cognitive failure associated with AD (Prasansuklab & Tencomnao, 2013).