UNIVERSITI TEKNOLOGI MARA

PROBIOTICS MEDIATE NEUROPROTECTION AGAINST AD THROUGH CNS AND ENS CROSSTALK: MODULATION OF NEUROTRANSMITTERS, NEUROPEPTIDES AND GUT HORMONES

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Alzheimer's disease (AD) is the commonest form of dementia characterised by aggregation of amyloid beta (Aβ) plaque and Tau protein. This neurodegenerative disease is now linked to altered gut microbiota composition which could result in increased permeability of the gut barrier and immune activation, leading to systemic inflammation, impaired blood-brain barrier, neuroinflammation, neural injury, and ultimately neurodegeneration. The limitations of current AD therapy, which mainly aims to alleviate symptoms rather than curing the disease, have called for alternative approach. The emergence of the Gut-Brain Axis concept raises possibility of using probiotics as neuroprotective agents. As such, the present study investigated the correlation between probiotic-induced neuroprotection and changes in composition of gut microbiota. For this purpose, lipopolysaccharide (LPS)-challenged rats characterised by neuroinflammation that mimic AD were used. The rats were subjected to the Morris Water Maze (MWM) Test, immunohistochemistry (IHC), microbiota diversity analyses, biochemical analyses and mitochondrial enzymatic analyses. LPS-challenged rats pre-treated with either LAB6 or LAB12 exhibited significantly (p<0.05) reduced escape latency and escape distance. They remained longer in the platform quadrant when compared to control. In IHC, LPS-challenged rats were presented with significantly (p<0.001) reduced 5-HT in hippocampi when compared to the wild type group. Pre-treatment with LAB significantly (p<0.01) restored 5-HT level by at least 37% when compared to the LPS control group. A similar trend was observed as 5-HT in colons of LAB pre-treated LPS-challenged rats was significantly (p<0.01) increased (+59%) when compared to the LPS control group. This study went on to explore the impact of LAB pre-treatment on compositional shift of gut microbiota. Bacterial 16S rRNA gene from caecal content was amplified using 515F-806R primers. LAB12, in particular, significantly (p<0.001) increased Bacteroidetes to Firmicutes ratio when compared to LPS control. Principal Component Analysis plot showed clear separation of bacteria community between LAB12 and LPS control groups. In order to further understand the role of LAB on gut microbiota and cognitive function, LPS-challenged rats were being administered with antibiotic cocktails (imipenem, vancomycin, ampicillin, ciprofloxacin and metronidazole) to mimic a close to germ free condition. The rodents were divided into groups (n=8/ group) of wild-type, LPS control, LAB12+LPS, ABX (antibiotics+LPS) and ABXL (ABX+LAB12). It was found that ABXL increased the time spent in the platform zone (+16%) as opposed to their ABX counterparts. In terms of mitochondrial enzymes, ABXL group were presented with increased Complex III enzyme activities in their cortices (+57 %; p<0.05) and hippocampi (+33 %; p<0.01) when compared to ABX group. In terms of neurotransmitters, ABXL group significantly increased 5-HT level (+32%, p<0.05) in the hippocampi when compared to ABX group. The ABXL group also significantly increased GHRL level (+85%, p<0.05), a gut hormone, in the hippocampi when compared to ABXL. Nevertheless, ABXL did not bring about significant changes against neuropeptides [neurotensin (NT), neuropeptide Y (NPY) and vasoactive intestestinal peptide (VIP)] in both hippocampi and colons. The present findings indicated that LAB neuroprotection could be mediated via crosstalk between the CNS and the ENS through modulation of neurotransmitter, gut hormone and compositional shift of gut microbiota

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