UNIVERSITI TEKNOLOGI MARA

INHIBITORY EFFECTS OF MALAYSIAN FUNGAL ENDOPHYTIC EXTRACTS ON BACE1 (β-secretase) AND BV2 MICROGLIA-MEDIATED INFLAMMATORY RESPONSE

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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 result 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 other 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

BACE1 is the major β-secretase in neurons and its appearance and activity were found to be elevated in the brain of Alzheimer's disease patients. In the present study, fungal endophytic extracts of BACE1 inhibitory activity and cytotoxicity against PC-12 (a rat pheochromocytoma with neuronal properties) and WRL68 (a non-tumorigenic human hepatic) were investigated. This study also investigated the anti-neuroinflammatory activities of five endophytic extracts (HAB16R12, HAB16R13, HAB16R14, HAB16R18 and HAB8R24) for inhibition of nitric oxide (NO), CD40 phenotype and pro- and anti-inflammatory cytokine production in lipopolysaccharide (LPS)-stimulated BV2 microglia cells. Endophytes were isolated from plants collected from Kuala Pilah, Negeri Sembilan and the National Park, Pahang. For investigation of biological activity, the pure endophytic cultures were cultivated for 14 days on PDA plates at 28°C and underwent semipolar extraction with ethyl acetate. From 212 endophytic extracts (1000 µg/ml), 29 exhibited more than 90% inhibition of BACE1 in the preliminary screening. Four extracts from isolates HAB16R13, HAB16R14, HAB16R18 and HAB8R24 identified as *Cytospora rhizophorae* were the most active with $IC_{50(BACE1)}$ values of less than 3.0 µg/ml. The most active extract HAB16R13 was shown to non-competitively inhibit BACE1 with K_i value of 10.0 µg/ml. HAB16R13 was considered non-potent against PC-12 ($IC_{50(CT)}$ of 60.0) and WRL68 ($IC_{50(CT)}$ of 40.0 µg/ml). Microglia treated with the five endophytic extracts (0.1 mg/ml) exhibited reduction of NO production without causing any effect on cell viability. CD40 expression in LPS-stimulated microglia was not significantly different in the presence or absence of endophytic extracts. In unstimulated BV2 cells, only extract HAB16R13 significantly ($p<0.05$) reduced CD40 expression after 48 h. All five extracts significantly ($p<0.05$) inhibited expression of the proinflammatory cytokines, IL-6 and TNF-α in LPS-stimulated microglia. This first report on endophytic fungal extract with BACE1 inhibitory activity and anti-neuroinflammatory properties demonstrates that more extensive study is required to uncover the potential of endophytes and could be utilized in the treatment of neurodegenerative diseases.

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