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NEUROPROTECTIVE EFFECT OF VIRGIN COCONUT OIL ON LIPOPOLYSACCHARIDE-INDUCED CELL DEATH IN SK-N-SH AND MEMORY IMPAIRMENT IN RAT

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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 degree 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

Neuroinflammation has been implicated in the pathogenesis of Alzheimer's Disease (AD) and often characterized by activation of glial cells and the subsequent up-regulation of various cytokines. Neuronal damage would then set in and lead to deterioration of cognitive function. Virgin Coconut Oil (VCO) has been reported to possess anti-bacterial, anti-viral, anti-oxidants and anti-inflammatory properties. Capitalizing on these therapeutic effects, the present study investigated for the first time the potential neuroprotective effect of VCO *in vitro* and *in vivo*. For this purpose, neuroprotection by VCO against amyloid-β-(Aβ) and Lipopolysaccharide(LPS)-induced cell death and Reactive Oxygen Species (ROS) production of SK-N-SH (neuroblastoma cells) was assessed. The *in vitro* findings were validated using normal and LPS-induced memory impaired animal models *in vivo*. A total of 36 male Wistar rats (7-8 weeks) were randomly assigned to 6 groups (n=6/group). The treatment groups were administered with 1, 5 and 10mL/kg of VCO for 31 days by oral gavages. The cognitive functions of the treated-rats were then assessed using the Morris Water Maze Test. Collected brains were homogenised and subjected to biochemical analyses of Acetylcholine (ACh), Acetylcholinesterase (AChE), antioxidative enzymes [Superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH), Glutathione peroxidase (GPx) and Glutathione reductase (GRx)], lipid peroxidase [Malondialdehyde (MDA)], nitric oxide (NO), cytokines (IL1-β, IFN-γ and IL-10) as well as inflammatory (COX-2 and iNOS) and amyloidogenic genes (BACE-1). Next, yet another 48 male Wistar rats (7-8 weeks) were assigned for neuroinflammatory study. The treatment groups (1, 5 and 10ml/kg) were administered with 1, 5 and 10mL/kg of VCO for 31 days by oral gavage in the presence of 0.25 mg/kg LPS (i.p.). α-Tocopherol (150 mg/kg) was used as positive control throughout the *in vivo* studies. The results showed that 100µg/mL VCO significantly improved viability of SK-N-SH (+47.25%; $p<0.01$ and 57.46%; $p<0.001$) and inhibited ROS production (-18.36%; $p<0.001$ and -30.96%; $p<0.001$) in the presence of Aβ and LPS respectively. Subsequent validation in normal rats indicated that VCO significantly enhanced cognitive functions [escape latency (-29.62±1.22%), escape distance (-23.87±0.20%) and total time spent on platform (+36.84%; $p<0.05$)]. The findings were mediated through elevation of ACh (+15.39%; $p<0.001$), SOD (+8.30%; $p<0.05$), CAT (+67.15%; $p<0.001$), GSH (+30.45%; $p<0.001$) and GPx (+14.31%; $p<0.001$) and reduction of AChE (-23.16%; $p<0.001$), MDA (-45.14%; $p<0.001$) and NO (-65.38%; $p<0.001$). On the other hand, exposure of LPS-induced memory impaired rats to VCO resulted significantly improved cognitive functions [escape latency(-45.87±0.61%), escape distance (-32.63±0.24%) and total time spent on platform (+81.82%; $p<0.01$)]. The improvements were mediated through elevation of ACh (+26.80%; $p<0.001$), SOD (+8.25%; $p<0.05$), CAT (+69.50%; $p<0.001$), GSH (+99.27%; $p<0.001$), GPx (+15.10%; $p<0.001$), GRx (+26.40%; $p<0.001$) and IL-10 (+34.05%; $p<0.01$) and reduction of AChE (-44.83%; $p<0.001$), MDA (-58.73%; $p<0.001$), NO (-52.71%; $p<0.001$), IL-1β (-64.85%; $p<0.001$), IFN-γ (-25.03%; $p<0.05$), COX-2 (-67.73%; $p<0.001$), iNOS (-65.63%; $p<0.001$) and BACE-1 (-75%; $p<0.001$). The present findings strongly implied that VCO has neuroprotective effects and has the potential to be a memory enhancer. This neuroprotective effect was mediated, at least in part, through the inflammatory, cholinergic and amyloidogenic pathways.

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CHAPTER ONE
INTRODUCTION

Neurodegenerative diseases occur when neurons in the brain or spinal cord begin to deteriorate. Changes in these cells cause them to function abnormally and eventually result in cell death. The progression of this condition will lead to impairment in memory, judgement, decision making and language ability (Thies & Bleiler, 2011). Alzheimer's disease (AD) is the most well-known neurodegenerative disease without cure (Choi, Lee, Hong, & Lee, 2012). In fact, it is the leading cause of dementia (especially among the elderly population) (Um, Ahn, Kim, & Ha, 2012) characterized by progressive memory loss and deterioration in cognitive abilities (Lemere & Masliah, 2010). In 2013, about 35 million people worldwide were living with AD. The number is expected to double by 2030 and more than triple by 2050 reaching 115 million people worldwide (Prince, Guerchet, & Prina, 2013). In Malaysia, about 50,000 people are suffering from AD (ADFM, 2014). The number, however, could be higher given that not all are officially diagnosed. The continuous rise of AD has brought about huge economic and personal burden to current and future generations both through direct (medical and social care) and indirect (care giving by families and friends) cost (Wimo, Jönsson, Bond, Prince, & Winblad, 2013).

The pathogenesis of AD remains poorly understood. Nevertheless, excessive aggregation of β-amyloid peptide (Aβ) has been found to be associated with the development and progression of AD (Dhanasekaran, Holcomb, Hitt, Tharakan, Porter et al., 2009). Aβ is a peptide derived from proteolysis of amyloid precursor protein (APP). Recently, several lines of evidence have further uncovered the correlation between oxidative stress and pathogenesis of AD (Zhang, Yu, Zhao, Lin, Tan et al., 2010). It was found that Aβ induces oxidative stress by causing mitochondrial dysfunction that may in turn result in increased reactive oxygen species (ROS). ROS is known to not only oxidise vital cellular components but also alter several signalling pathways including apoptosis by modulation of Bcl-2 and p53 protein (Brunet, Datta, & Greenberg, 2001; Dypbukt, Ankarcrona, Burkitt, Sjöholm, Ström et al., 1994). As such, excessive production of ROS can cause cellular damage and subsequently cell death.