UNIVERSITI TEKNOLOGI MARA

CHARACTERIZATION OF NANOEMULSION FROM ETHANOLIC EXTRACT OF *CENTELLA ASIATICA* (NANOSECA) FOR MEMORY AND COGNITIVE ENHANCEMENT

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ABSTRACT

The evidence of the neuroprotective impact of *Centella asiatica* (*C. asiatica*) has been documented in recent years. It is a medicinal plant that possesses extensive antioxidant, anti-inflammatory and anti-acetylcholinesterase (AChE) activities. However, a major obstacle that remains to be overcome is the capacity of the active molecules in *C. asiatica* to cross the blood-brain barrier (BBB). Therefore, the present study developed a nanoemulsion from selected ethanolic extract of *C. asiatica* to improve brain bioavailability. *C. asiatica* accessions (CA-K017, CA-K018, CA-K019) were characterized and extracted, designated as SECA-K017, SECA-K018, SECA-K019. In addition, major triterpenes of SECA were identified and quantified using high-performance liquid chromatography (HPLC). D-optimal mixture design was used to determine the optimal oil, water, and surfactant concentration. In this view, the biological activities of triterpenes, SECA accessions and NanoSECA were explored via *in vitro* (SH-SY5Y and RAW 264.7 cells) system. The antioxidant and anti-inflammatory activities were also evaluated *in vitro* using lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. Meanwhile, anti-acetylcholinesterase (AChE) was investigated using Ellman's Spectrophotometric Method. Moreover, molecular docking was performed on active constituents of SECA. The prediction of substance permeability across biological membrane systems was evaluated using a parallel artificial membrane permeability assay (PAMPA). *In vivo,* experimental procedures were conducted on acute and sub-acute toxicity, neurobehavioral assessment, and preliminary pharmacokinetic and pharmacodynamic (PK/PD) study. The potential of NanoSECA in improving memory and cognitive functions was explored in the neurobehavioral task (Morris Water Maze), and biochemical assays AChE and ACh, antioxidant (glutathione; GSH and malondialdehyde; MDA) and anti-inflammatory (nitrite; NO, tumor necrosis factor-alpha; TNF-α and prostaglandin E_2; PGE_2). Results revealed that all accession contains a higher proportion of glycosides than the aglycones, with madecassoside in the highest proportion, followed by asiatic acid. It was found that the optimized NanoSECA has a particle size of 127.833 ± 8.280 nm, zeta potential (ZP) of -24.9 ± 0.011 m V, and polydispersity index (PDI) of -0.493 ± 4.681. All tested samples showed no toxicity effect at the concentration tested since its IC_{50} could not be determined in a concentration ranging from 7.8125 to 1000 µg/mL. Results revealed that treatment with NanoSECA significantly suppressed reactive oxygen species and moderately attenuated the nitrite and AChE production. This further reveals that madecassic acid and asiatic acid prevented acetylcholine (ACh) molecules from interacting with binding site residues, thus obstructing the hydrolysis reaction. This would cause an elevated ACh concentration, eventually increasing cholinergic transmission. NanoSECA tended to exhibit higher blood-brain barrier (BBB) permeation values (Pe: 15.19 ± 0.3 10^{-6} $cm.s^{-1}$). Acute toxicity study revealed non-toxic of NanoSECA until a dose of 2000 mg/kg. However, a sub-acute toxicity study showed no mortality but mild congestion in several organs. Findings revealed that NanoSECA could be used as a memory enhancer through cholinergic activity, increased anti-oxidant levels, and reduced oxidative stress. On the other hand, the analytical method for NanoSECA administration was well developed and validated.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Healthy aging is accompanied by numerous functional and structural brain changes that contribute to age-related cognitive decline (Seevaratnam *et al.,* 2012; Koen *et al.,* 2019). Aging-related losses are understood as consequences of the progressive damage of living tissues due to chromosomal damage, accumulation of mutations in Deoxyribonucleic acid (DNA), and molecular damage caused by oxidative stress (Morterá & Herculano-Houzel, 2012).

While there have been several reports of age-related changes in cognitive functioning over the last 100 years, the age at which cognitive impairment starts remains a source of debate. Currently, many programs are aimed only at people aged 60 and above. However, some forms of age-related cognitive impairment can now be seen in healthy, trained emerging adults in their 20s and 30s (Salthouse, 2009). Therefore, the potential causes of cognitive decline before the age of 60 were investigated. In addition, cognitive dysfunction is linked to increased oxidative stress susceptibility of various biological components (e.g., lipids, proteins, nucleic acids, and cholinergic neurotransmitters) (Papandreou *et al.,* 2009). Therefore, preventing or delaying the onset of age-related cognitive deterioration will significantly reduce the likelihood of functional disability.

Cognition is the mental process of acquiring knowledge and understanding, problem-solving, language, creativity, enabling critical thinking, and mastering fundamental concepts (Foster *et al.,* 2019). Learning is the process of gaining new information about the world and environment, while memory is storing or retaining that information. Memory refers to a person's ability to record sensory sensations, events, and news, maintain that information for a short or long period, and recall it when necessary (Aqilah *et al.,* 2018). Several brain regions are involved in learning and memory, including the cortex, amygdala, cerebellum, and hippocampus. From memory creation to memory retrieval, several processes take place, including encoding, memory storage, consolidation, and recall. Long-term potentiation (LTP) and synaptic plasticity are also involved in memory development, with the hippocampus becoming stimulated