UNIVERSITI TEKNOLOGI MARA

COMPARATIVE STUDY ON NEUROPROTECTIVE EFFECTS OF SOYBEAN AND *TEMPEH* EXTRACTS

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AUTHOR'S DECLARATION

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

Hereby, I acknowledge that I have been supplied with the Academic Rules and Regulations for Postgraduate, Universiti Teknologi MARA, in which regulating the conduct of my study and research.

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ABSTRACT

Although there were several studies regarding the benefits of soybean to health, only a few focused on neuroprotective effects. Furthermore, the role of fermented soybean has not been documented as neuroprotective agent. The present study is therefore aimed to compare the neuroprotective effects of total isoflavones from soybean and *tempeh* extracts. Total isoflavones were extracted from soybean and *tempeh*. The methanolic extracts were subjected to HPLC analysis to quantify the amount of isoflavones present in soybean and *tempeh*. The animal study was set up for two models as normal and scopolamine-induced (dementia model). The rats (n=6) were given oral administration of soybean and *tempeh* extracts (10, 20 and 40 mg/kg), piracetam (400 mg/kg) as standard and normal saline as control for 15 days. Both models were tested for radial arm maze task (RAM) and elevated plus maze (EPM) task for the measurement of memory and learning behavior. On 30^{th} day, after the behavior study and treatment, the animals were sacrificed. The brain and serum were collected for superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione reductase (GR), thiobarbituric acid (TBARS), nitric oxide (NO), acetylcholine (ACh) and acetylcholinesterase (AChE) measurement. For neuroinflammatory analysis, two cytokines (IL-10 and IL-1β) were also measured. The HPLC analysis showed that *tempeh* possessed higher aglycone level which indicated higher bioavailability as compared to soybean glycoside. The results of the *in vitro* studies highlighted that *tempeh* extract exerted more radical scavenging effect and ferrous ion chelating as compared to soybean extract. For β-secretase (BACE1) inhibition, *tempeh* extract also showed a lower IC_{50} value as compared to soybean extract although not enough evidence to statistically significant. *In vivo* memory models, showed significant improvement of memory enhancing by both extracts as compared to control in both normal and scopolamine-induced models. Significant values ($p < 0.05$) were higher in *tempeh* treated groups. Similarly, the improvement of antioxidants SOD, CAT, GSH, GR and reduction in oxidative parameters LPO and NO were higher in *tempeh* compared to control. Furthermore, higher cholinergic activity such as improved the Ach level and declined the AChE activity were observed in *tempeh* as compared to control. For the inflammatory study, IL-10 was significantly up-regulated while IL-1β was significantly down-regulated in both soybean and *tempeh* treated group as compared to control and scopolamine group. This suggested soybean and *tempeh* as a beneficial food for anti-inflammation within the brain. As a conclusion, *tempeh* extract exerted higher neuroprotective activities than soybean. Hence, consumption of *tempeh*, is more health beneficial than soybean.

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CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF STUDY

Neurodegeneration is a series of neuronal dysfunction due to the continual death of neuron. The loss of brain neurons leads to major age-related disease like dementia. The most common type of dementia is Alzheimer's disease (AD), while other reported types of dementia are Parkinson's disease, Huntington's disease, dementia with Lewy bodies, frontotemporal dementia, Korsakoff's syndrome, Creutzfeldt-Jakob disease, HIV-related cognitive impairment, mild cognitive impairment and vascular dementia (Wimo & Prince, 2010).

The present study is focused more on AD. This disease is characterised by loss of memory, incapability to learn, communicate, and make judgements (Bagheri, Joghataei, Mohseni and Roghani, 2011). The major neuropathological hallmarks of AD are the aggregation of β-amyloid (Aβ) plaques and neurofibrillary tangles (NFT). This includes high level of free radicals (superoxide and nitric oxide) (Butterfield, Reed, Newman and Sultana, 2007; Butterfield *et al.,* 2010), continuous lipid peroxidation (Butterfield, Castegna, Lauderback and Drake, 2002; Tanea, 2011), cholinergic dysfunction (Lee *et al.,* 2009) and also neuroinflammation (Heneka & O'Banion, 2007).

Recent studies have focused on the correlation between AD and its related pathological factors. Studies suggested that the accumulation of Aβ plaques were involved in increasing the production of free radicals. These free radicals further reacted with other metabolites in brain such as lipid, which resulted in the production of lipid peroxidation and damaged to the cells/neurons membrane. Other indigenous self defence enzymes such as superoxide dismutase (SOD), catalase (CAT) (Clausen, Doctrow and Baudry, 2010), glutathione (GSH), glutathione reductase (GR) and glutathione peroxidase (GPX) (Vina, Lloret, Orti and Alonso, 2004) are also reduced in the present of highly reactive free radicals in the system. The deficiency in the oxidative defence enzymes may harm the neurons and uncontrollable situation leads to cell death.

Cholinergic imbalance may also contribute to AD. It includes the high level of acetylcholinesterase and low level of acetylcholine. High level of acetylcholinesterase results in the degradation of acetylcholine, a neurotransmitter which is crucial in the