

Anticholinesterases Potential of Three Thai Colored Rice Extracts in Preventing Alzheimer's Disease

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Abstract

The purpose of this study was to explore acetylcholinesterase and butyrylcholinesterase activities of three Thai colored rice extracts including Chiang Mai local black rice, Kum Doi Saket and Riceberry. Cholinesterases inhibitors could improve some symptoms and slow down the progression of Alzheimer's disease. In this study, cholinesterases activities were determined using modified Ellman's colorimetric method. The results demonstrated that all extracts exhibited inhibitory effect on dual enzymes. Interestingly, the data indicated more inhibition on butyrylcholinesterase than acetylcholinesterase. These findings suggested that Thai colored rice may be utilized as a natural prevention for Alzheimer's disease and other related dementias.

Keywords : Anticholinesterases, AChE, BuChE, colored rice, Alzheimer's disease

Introduction

Alzheimer's disease (AD) is a chronic progressive neurodegenerative disorder. The symptoms of AD are difficulties with memory, language, problem-solving and other cognitive skills that affect a person's ability to perform everyday activities. Recently, AD is one of the most common cause of dementia with severe economic and social impacts (Alzheimer's Association, 2017: 325-373). Based on cholinergic hypothesis, the loss of cholinergic neurons that decreases acetylcholine (ACh) level could lead to deterioration in cognitive function (Hoffman Snyder and Facchiano, 2011: 201-206). Cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), are involved in the metabolic hydrolysis of ACh. They play the key role in ending cholinergic neurotransmission. Consequently, the inhibition of both enzymes could increase the duration of action as well as the concentration of ACh can be thought as a promising approach for the control of AD (Mushtaq et al., 2014: 1432-1439 and Saify and Sultana, 2014: 387-425). In this study, three Thai colored rice extracts including Chiang Mai local black rice, Kum Doi Saket and Riceberry which are known for their antioxidant properties and play a crucial role for memory enhancement (Muntana, 2010: 170 and Pengkumsri, 2015: 331-338 and Settapramote, 2018 and Walter and Marchesan, 2011: 371-377) were investigated for the AChE and BuChE inhibitory activities using modified Ellman's colorimetric method.

Materials and methods

Chemicals Three Thai colored rice varieties including Chiang Mai local black rice (CM), Kum Doi Saket (KD) and Riceberry (RB) were collected from Chiang Mai, Thailand. AChE from *Electrophorus electricus* (*EeAChE*) (E.C.3.1.1.7, Sigma C2888), BuChE from equine serum (*EqBuChE*) (E.C.3.1.1.8, Sigma C7512), acetylthiocholine iodide (ATCh), butyrylthiocholine iodide (BTCh), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), bovine serum albumin (BSA) and the reference compound, donepezil hydrochloride (donepezil HCl), were purchased from Sigma-Aldrich Chemical (St. Louis, MA, USA). Tris(hydroxymethyl)aminomethane was acquired from Merck Millipore Corporation,

Calbiochem (San Diego, CA, USA). Hydrochloric acid (HCl) and ethanol were purchased from commercial suppliers.

Sample preparation and extraction Firstly, impurities of all samples were detected and sorted out effectively. Then all hulls were removed by milling to obtain brown rice. After that, they were dried and grinded into powder before extraction. Then, each powdered sample was extracted using 15% w/v of 0.1 N HCl in 95% ethanol as solvent. The ratio between solvent and solid material was 1:10. Each sample was mixed on magnetic stirrer at 500 rpm for 30 minutes, and then was filtered with Whatman No.1 filter paper. The extraction process was repeated 3 times. Finally, the filtrate of each sample was evaporated to dryness at 50°C with vacuum rotary evaporator and freeze dryer.

Anticholinesterases test An assessment of *in vitro* AChE and BuChE inhibition were tested in conventional, flat-bottomed 96-well microplates (Thermo Fisher Scientific, Waltham, MA, USA) using the modified Ellman's method (Ellman et.al, 1961; 88-95 and Miao and Zhu, 2010: 5216-5234). The reaction mixture consisted of 25 µL of tested compound solution, 25 µL of 15 mM ATCh or 1.5 mM BTCh, 50 µL of 0.1% w/v bovine serum albumin in 50 mM Tris(hydroxymethyl)aminomethane pH 8, 125 µL of 3 mM DTNB and 25 µL of 0.22 units/mL *Ee*AChE or 0.50 units/mL *Eq*BuChE. The final volume of this test was 250 µL. After 20 minutes, the reaction was incubated at 25°C and the absorbance was measured at 405 nm from microplate reader UV scan (EZ read 2000 microplate reader, Biochrom, UK). As a control, the tested compound solution was replaced with solvent that used for dissolving sample. The control and tested samples were performed in triplicate. To monitor any nonenzymatic hydrolysis in the reaction mixture, two blanks for each analysis were prepared in triplicate. Percentage of AChE or BuChE inhibition was calculated by the following equation;

$$\% \text{ Inhibition} = \frac{(A-B) - (C-D)}{A-B} \times 100 \quad (1)$$

where A is absorbance of control,
B is absorbance of blank,
C is absorbance of sample,
D is absorbance of sample blank

In this experiment, donepezil HCl with the concentration from 0.0625 to 10 µg/mL was used as the reference compound. All results were calculated as the percentage inhibition. The results from all samples were reported as donepezil equivalent (DNPZE).

Statistical analysis Statistical significance was determined using one-way analysis of variant (ANOVA) with LDS post hoc analysis at 95% confidence interval using SPSS Statistics v17.0 program.

Results

Extracts of three Thai colored rice varieties including Chiang Mai local black rice (CM), Kum Doi Saket (KD) and Riceberry (RB) were evaluated anticholinesterases activities including AChE and BuChE using modified Ellman's colorimetric method in microplate. The results of these extracts were calculated as donepezil equivalent (DNPZE). The results of these activities are shown in Figure 1.

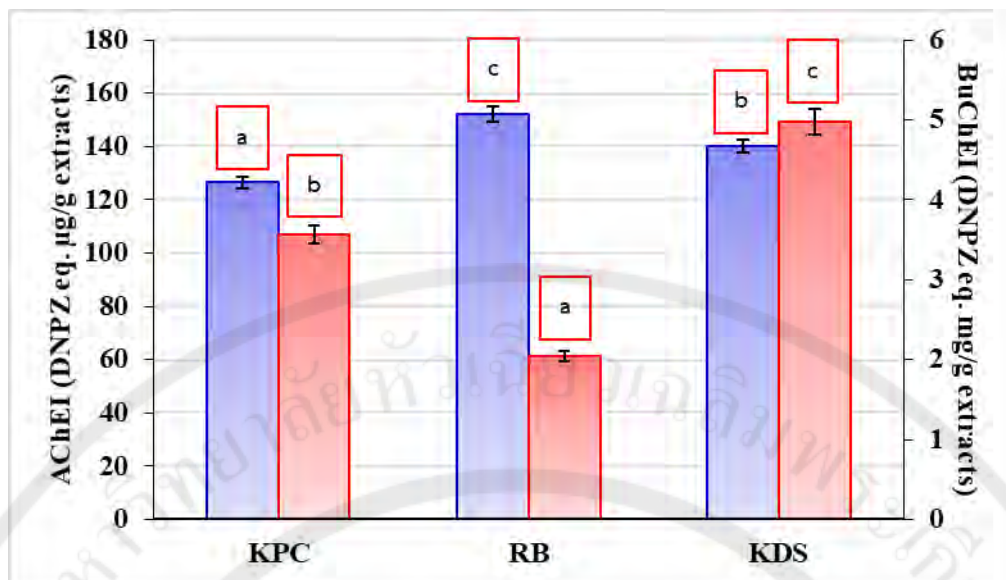


Figure 1 Anticholinesterases activities of Chiang Mai local black rice (CM), Kum Doi Saket (KD) and Riceberry (RB) extracts. All data are represented as mean \pm SD, $n = 3$. a, b and c are the different significant at 95% CI using ANOVA test with Turkey post hoc analysis.

For anti-AChE study, RB extract demonstrated the highest inhibitory activity with DNPZE value at $152.05 \pm 2.84 \mu\text{g/g}$ extract. KD and CM extracts could also inhibit this enzyme with DNPZE value at 140.16 ± 2.61 and $126.44 \pm 2.36 \mu\text{g/g}$ extract, respectively. For anti-BuChE study, KD extract exhibited the greatest inhibitory activity with DNPZE value at $4.09 \pm 0.16 \text{ mg/g}$ extract. CM and RB extracts also showed the inhibitory effect with DNPZE value at 2.73 ± 0.11 and $3.66 \pm 0.06 \text{ mg/g}$ extract, respectively. A one-way ANOVA expressed significant difference values at 95% confidence interval among the different samples in both studies.

Discussion and conclusion

In this study, we focused on anticholinesterases properties of Thai colored rice extracts, Chiang Mai local black rice (CM), Kum Doi Saket (KD) and Riceberry (RB). It has been found that that all of them could inhibit not only AChE but also BuChE. However, the data revealed more inhibition of BuChE than AChE. These results were similar to earlier report (Kukreja et al., 2018). These may be described in term of substrate binding cavity. It has been previously found that the catalytic pocket of BuChE is larger than AChE (Bajda et al., 2013). Therefore, several inhibitors could selectively bind to the active site of BuChE as being observed in this research. Furthermore, it has been reported in the literature that cholinesterase inhibitors inhibit both AChE and BuChE as well as highly selective BuChE inhibitors may have potential therapeutic benefits in the treatment of Alzheimer's disease and other related dementias (Mushtaq et al., 2014: 1432-1439). According to the results stated above, it has been suggested that Thai colored rice may be one of natural source for healthy consumptions to prevent Alzheimer's disease.

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