Acetyl and butyryl-cholinesterase inhibitory activity of bran extracts of some Sri Lankan traditional red rice (*Oryza sativa* L.)

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Introduction

A prevalent therapeutic approach in treating neurodegenerative diseases such as Alzheimer's disease is to enhance acetylthiocholine (ACh) levels using inhibitors of acetylcholinesterase (AChE), the selective enzyme responsible for the hydrolysis of ACh at the cholinergic synapses (Lawrence and Shakian, 1998). In addition to AChE, a substrate non-specific butyrylcholinesterase (BChE) also plays an important role in hydrolyzing ACh (Greig *et al*., 2002). Therefore, cholinesterase inhibitors intended for treatment of Alzheimer's disease should have a good inhibition towards both enzymes. Role of oxidative stress in neurodegeneration is well reported and several antioxidant rich natural products are reported to have cholinesterase enzyme inhibitory activity (Pannangrong *et al*., 2011). Previously we reported the marked antioxidant activities of brans of selected Sri Lankan traditional red rice. The present study evaluates the acetyl and butyryl-cholinesterase inhibitory activity of brans of these selected rice varieties (RV) *in vitro*.

Materials and Methods

Four Sri Lankan traditional red rice varieties (Sudu Heeneti, Goda Heeneti, Masuran and Dik Wee) were obtained from Rice Research and Development Institute (RRDI), Batalagoda. Rice seeds were dehulled (Satake THU 35B), polished in a laboratory mill (Satake TM 05C) and passed through a 60-mesh sieve, resulting in a uniform fraction of rice bran. Rice brans were then extracted by shaking for overnight at room temperature with 10 times the sample weight of 70 % ethanol-water (v/v). Rice extracts were centrifuged and filtered through 0.45 µm nylon filters. Crude rice extracts obtained by filtration were evaporated to dryness with a rotary evaporator, under reduced pressure at 40 ºC and freeze dried. The freeze dried extracts were subjected to *in vitro* acetylcholinesterase/butyrylthiocholinesterase enzyme inhibition assays.

Acetylcholinesterase/butyrylthiocholinesterase enzyme inhibition assay

AChE and BChE inhibition assay was performed according to method of Ellman et al. (1961) with some modifications using 96 well micro plates. A reaction volume of 200 µl containing 150 µl of 0.1 M sodium phosphate buffer (pH 8.0), 0.02 mU of AChE/ BChE (10 µl) and 10 µl of different concentrations of rice bran extracts and the positive control

were pre incubated for 15 min at 25 °C. The reaction was then initiated by the addition of 0.071 mM acetylthiocholine/butyrylthiocholine and 0.5 mM DNTB in 20 µl of 0.1 M sodium phosphate buffer. The hydrolysis of acetylthiocholine/butyrylthiocholine was monitored by the formation of yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine for a period of 10 min at 412 nm using 96 well micro plate reader. Galanthamine was used as the positive control. Rice bran extracts were dissolved in 50 % ethanol. Control incubations were carried out in the same way while replacing rice bran extracts with same amount of 50 % ethanol. All the reactions were performed in triplicate. The kinetic parameter *V*max was used to calculate the % inhibition. The concentrations of rice bran extracts and the positive control that inhibited the hydrolysis of acetylthiocholine/butyrylthiocholine by 50 % (IC_{50}) were calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, MA, U.S.A.).

Results and Discussion

Acetylcholinesterase and butyrylthiocholinesterase enzyme inhibitory activity of brans of selected Sri Lankan traditional rice is given in Table 1 and 2.

Table 1: Acetylcholinesterase inhibitory activity of selected Sri Lankan traditional red rice

	Concentration (µg/ml)				
Rice variety	**25**	**50**	**100**	**200**	**IC_{50} (µg/ml)**
Sudu Heeneti	44.36 ± 0.39	58.79 ± 0.45	66.55 ± 0.04	68.26 ± 0.55	37.00 ± 0.68
Dik Wee	37.28 ± 1.27	47.86 ± 1.33	56.56 ± 1.42	63.15 ± 0.42	77.03 ± 2.37
Masuran	27.75 ± 0.72	44.71 ± 3.77	52.16 ± 0.39	58.60 ± 0.18	88.27 ± 6.64
Goda Heeneti	17.51 ± 0.39	26.09 ± 0.72	33.52 ± 0.39	39.30 ± 1.42	291.00 ± 3.54

Data represented as mean ± SE (n=3). IC_{50} values superscripted by different letters are significantly different at $p < 0.05$; IC_{50}: Galanthamine: 0.46 ± 0.02 µg/ml

Results revealed that bran extracts of all the selected rice varieties with both enzyme inhibitory activities. However, significant differences were observed among the varieties too for both enzymes ($p<0.05$). Bran extract of Sudu Heeneti demonstrated significant ($p<0.05$) and highest enzyme inhibition compared to other varieties for both enzymes and IC_{50} values for AChE and BChE were 37.00 ± 0.68 and 18.50 ± 0.60 µg/ml respectively. The order of potency of enzyme inhibition exerted by bran extracts of selected RV followed the similar pattern for both enzymes as SH>DW>M>GH. IC_{50} values of DW, M and GH for AChE and BChE were 77.03 ± 2.37, 88.27 ± 6.64, 291.00 ± 3.54 and 30.33 ± 0.32, 30.20 ± 1.96, 96.60 ± 0.56 µg/ml respectively. Results revealed that inhibition of BChE are more prominent compared to AChE inhibition. This is the first report of acetylcholinesterase and butyrylcholinesterase enzyme inhibitory activity by bran extracts from Sri Lankan rice varieties.

Table 2: Butyrylcholinesterase inhibitory activity of selected Sri Lankan traditional red rice

	Concentration (µg/ml)				
Rice variety	**12.5**	**25**	**50**	**100**	**IC_{50} (µg/ml)**
Sudu Heeneti	39.84 ± 0.66	64.52 ± 1.40	79.80 ± 0.45	86.56 ± 0.57	18.50 ± 0.60
Dik Wee	30.27 ± 3.29	49.18 ± 0.10	69.74 ± 0.51	81.36 ± 0.98	30.33 ± 0.32
Masuran	25.11 ± 0.27	50.99 ± 1.99	65.75 ± 0.23	78.27 ± 1.16	30.20 ± 1.96
Goda Heeneti	18.11 ± 2.37	32.57 ± 4.59	41.91 ± 0.50	50.68 ± 0.18	96.60 ± 0.56

Data represented as mean ± SE (n=3). IC_{50} values superscripted by different letters are significantly different at $p < 0.05$; IC_{50}: Galanthamine: 3.03 ± 0.01µg/ml

Conclusion

It is concluded that, brans of these selected Sri Lankan traditional red rice may play an important role in managing neurodegenerative diseases such as Alzheimer's disease. Results also show the possibility of isolating active compounds from these selected rice bran and those compounds may have the potential for development of novel nutraceuticals and pharmaceuticals for managing Alzheimer's disease.

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