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Anti-diabetic related health food properties of traditional rice (*Oryza sativa* L.) in Sri Lanka

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ABSTRACT

Objective: To evaluate a range of anti-diabetic related properties and some consumer preferred physicochemical properties of selected Sri Lankan traditional rice varieties.

Methods: Sudu Heeneti, Goda Heeneti, Masuran and Dik Wee varieties were used in this study. Anti-diabetic related properties of bran extracts of selected varieties were studied for methylglyoxal mediated protein glycation inhibition, acetyl and butyryl-cholinesterase inhibition *in vitro* and anti-hyperglycemic activity *in vivo*. Further, selected varieties were studied for starch hydrolysis rate *in vitro*. Physicochemical properties including grain color, size, shape, crude protein, crude fat, ash, dietary fiber and total carbohydrate contents were studied.

Results: Brans of selected varieties had significant ($P < 0.05$) and dose dependent methylglyoxal mediated protein glycation inhibition [IC_{50}: (174.77 ± 6.65) to (342.87 ± 0.43) μg/mL] and acetyl [(IC_{50}: (37.00 ± 0.68) to (291.00 ± 3.54) μg/mL] and butyryl-cholinesterase [IC_{50}: (18.50 ± 0.60) to (96.60 ± 0.56) μg/mL] inhibitory activities. Further, Sudu Heeneti, Masuran and Dik Wee had low starch digestion rate (52.40 ± 1.44 to 53.76 ± 1.19) indicating that these varieties may be low glycemic index rices. Brans of Masuran tested in rat model showed anti-hyperglycemic activity. Physicochemical properties studied showed that selected varieties were red in color and grain size and shape were mostly medium and bold respectively. Moisture, crude protein, crude fat, ash and total carbohydrate contents varied significantly ($P < 0.05$) among the varieties.

Conclusions: It is concluded that selected varieties could be promoted as physicochemically sound rices with a range of anti-diabetic related properties in the management of diabetes and its complications.

1. Introduction

Diabetes mellitus is a chronic metabolic disease increasing rapidly in both developed and developing countries[1,2]. Major categories of diabetes include type 1 and type 2[1,2]. Recently, type 3 diabetes is termed and referred to Alzheimer's disease as it is a neuroendocrine disease associated with insulin signaling and harbors characteristics of both types 1 and 2 diabetes[3-5].

Currently, functional foods are rapidly becoming popular among health conscious consumers and highly priced over the other foods as they have the ability of prevention and management of chronic diseases[6,7]. Rice is one of the most important cereal crops worldwide[8]. It is the dietary staple for half of the world's population and widely cultivated in over 100 countries[8,9]. As rice is one of the most important food crops worldwide, identification of rice varieties having anti-diabetic related properties is important for prevention and management of diabetes and other related complications. Consumer preference of rice also depends on physicochemical properties of the rice grain[10]. Therefore, rice varieties with desirable physicochemical and anti-diabetic related properties may be increasingly becoming popular among health conscious consumers and can be highly priced in the international

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trade.

In Sri Lanka, rice is also the dietary staple and country holds many traditional rice varieties which had been in the diet for centuries. During early periods (up to 1950s), the rice varieties used for cultivation were exclusively these traditional varieties[11]. After the introduction of newly improved varieties, these traditional varieties were gradually disappeared mainly because of their relatively low yield potential[11]. However, recent research publications have shown that traditional rice varieties are much better in nutritional and other health properties compared to newly improved varieties[12-14]. Therefore, much attention has gained among policy makers, farmers, food scientists, nutritionists and other health professionals in the country for cultivation and promotion of these varieties. In Sri Lankan traditional and folk medicine also some of these varieties are claimed to possess variety of health properties including anti-diabetic properties[13]. We have recently reported amylase and glucosidase inhibitory activity, antioxidant activity, anti-glycation and glycation reversing properties of some of these Sri Lankan traditional rice varieties[14]. Globally, extremely limited studies have been attempted to investigate the effect of different rice varieties on type 3 diabetes. To the best of our knowledge, methylglyoxal (MGO) mediated protein glycation inhibition and butyryl-cholinesterase (BChE) inhibition by rice has not been previously reported. Therefore, present study was undertaken to evaluate the *in vitro* starch digestion rate (indication of glycaemic index), inhibition of MGO mediated protein glycation, *in vitro* acetyl and BChE inhibitory activities (related to type 3 diabetes), anti-hyperglycaemic activity and consumer preferred physicochemical properties for selected Sri Lankan traditional rice varieties.

2. Materials and methods

2.1. Grain samples

Sri Lankan traditional red rice varieties: Sudu Heeneti, Goda Heeneti, Masuran and Dik Wee were obtained from Rice Research and Development Centre (RRDC), Bombuwala, Sri Lanka. The selected rice varieties were cultivated and harvested under experimental field conditions at RRDC, Bombuwala and these varieties were currently very popular among farmers and health conscious consumers.

2.2. Chemicals and reagents

Pepsin (porcine stomach mucosa), pancreatin (bovine pancreas), heat stable alpha amylase, acetylcholinesterase (AChE) from electric eel (Type VI-S), BChE from horse serum, acetylthiocholine, butyrylthiocholine, 5,5′-dithiobis-(2-nitrobenzoic acid), bovine serum albumin, MGO, dimethyl sulfoxide (DMSO), rutin, galanthamine hydrobromide and dialysis tubing (12000 molecular weight cutoff) were purchased from Sigma-Aldrich, St. Louis, USA. All other chemicals and reagents were of analytical grade.

2.3. Preparation of grain samples

Rice seeds were dehulled (THU 35B, Satake, Hiroshima, Japan) and stored in airtight containers at 8 ˚C in a cold room until use for the analysis. Preparation of rice flour for the analysis was performed immediately before analysis by using an ultra centrifugal mill with a 0.25 mm sieve. The whole grains (brown rice) were used to prepare the rice flour and used for the physicochemical analysis.

2.4. Preparation of rice bran extracts

Whole grains were polished in a laboratory mill (TM-05C, Satake, Hiroshima, Japan) and passed through a 60 mesh sieve, to obtain a uniform fraction of rice bran. Rice brans of each of the rice varieties were extracted with 10 times the sample weight of 70% ethanol-water (v/v) overnight at room temperature [(28 ± 2) ˚C]. Rice bran extracts were then centrifuged (825 g) for 10 min, filtered through 0.45 μm nylon filters and evaporated to dryness under vacuum in a rotary evaporator and freeze dried (Christ Alpha 1-4 freeze dryer, Biotech International, Germany).

2.5. Anti-diabetic related properties

2.5.1. In vitro starch digestion rate

Two grams available carbohydrate portions of each of the cooked rice samples (whole grains) were placed in dialysis bags (12000 molecular weight cutoff), 10 mL of pooled human saliva was added and stirred for 10 s and the volume was adjusted to 35 mL with distilled water. Then samples were dialyzed against 800 mL of distilled water at 37 ˚C for 3 h (n = 4). White bread was used as the reference and digestion and the dialysis (n = 4) were carried out in the same manner. Hourly, for 3 h, 1 mL dialysate samples were taken and analyzed for total sugars by 3,5-dinitrosalicylic acid method[15].

2.5.2. MGO mediated protein glycation inhibitory activity

This assay was carried out according to the method of Lunceford and Gugliucci[16]. Reaction volume of 1 mL containing 1 mg bovine serum albumin, 5 mmol/L MGO and different concentrations of rice bran extracts (25, 50, 100, 200, 400 μg/mL) in 0.1 mol/L phosphate buffer (pH 7.4) was incubated at 37 ˚C for 6 days (n = 3). The test solution also contained 0.2 g/L NaN_3 to ensure an aseptic condition. After the incubation period, florescence was measured at an excitation of 370 nm and emission at 440 nm using 96 well florescence micro plate reader (SpectraMax Gemini EM, Molecular Devices, USA). Control experiments were conducted in an identical way replacing rice bran extracts with 0.1 mol/L phosphate buffer. For sample blank incubations, MGO solution was replaced with phosphate buffer and the same procedure was carried out. Rutin was used as the positive control. Inhibitory activity of each rice bran extract and rutin was calculated using following equation.

Inhibition (%) = $[A_c - (A_s - A_b)/A_c] \times 100$

where, A_c is the florescence of the control, A_b is the florescence of the sample blank and A_s is the florescence in the presence of rice bran extract or rutin.

2.5.3. AChE and butyryl-cholinesterase inhibitory activity

AChE and BChE inhibition assays were performed according to method of Bhadra *et al.* with some modifications using 96 well micro plates[17]. A reaction volume of 200 μL containing 150 μL of 0.1 mol/L sodium phosphate buffer (pH 8.0), 0.02 mU of AChE/

BChE (10 μL) and 10 μL of different concentrations of rice bran extracts (AChE: 25, 50, 100, 200 μg/mL; BChE: 12.5, 25, 50, 100 μg/mL) and the positive control were pre incubated for 15 min at 25 ˚C. The reaction was then initiated by the addition of 0.071 mmol/L acetylthiocholine/butyrylthiocholine and 0.5 mmol/L 5,5′-dithiobis-(2-nitrobenzoic acid). The hydrolysis of acetylthiocholine/butyrylthiocholine was monitored by the formation of yellow colored 5-thio-2-nitrobenzoate anion for a period of 10 min at 412 nm using 96 well micro plate reader (SpectraMax Plus 384, Molecular Devices, USA). Galanthamine was used as the positive control. Rice bran extracts were dissolved in 50% ethanol. Control incubations were carried out in the same way 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 and IC_{50} values were calculated using the EZ-FIT enzyme kinetics program (Perrella Scientific Inc., Amherst, MA, USA).

2.5.4. Anti-hyperglycemic activity

Male Wister rats obtained from Medical Research Institute, Colombo, Sri Lanka were used for the study. The animals were maintained on a commercially available standard pellet diet with free access to water at the animal house in the Department of Zoology, University of Colombo with control conditions: temperature [(25 ± 2) ˚C], humidity (50%–55%) and light (12 h natural light per day). This experiment was conducted in accordance with the internationally accepted laboratory animal use and care, and guidelines and the rules of the Department of Zoology, Faculty of Science, University of Colombo, Sri Lanka for animal experiments[18]. Rats were randomly divided into two groups (each group: n = 8) and fasted for 18 h with free access to water. The treatment group received 1 g/kg rice bran extract in 1.5 mL of 10% DMSO in distilled water. Ten percent DMSO served as the control. After 10 min, soluble starch (2 g/kg body weight) was administered orally for the two groups. Then, blood was collected from the tail of the rat and glucose levels were measured at 0, 15, 30, 60, 90, 120 and 180 min using a glucometer (Accu-Chek Active-Roche, Germany; standardize before used)[19-21].

2.6. Physicochemical properties of the rice grain

Grain color was determined by visual observation and grain size and shape were determined by internationally accepted standard methods[22,23]. Moisture, ash, crude fat and crude protein contents (n = 4 each) were determined according to Association of Official Analytical Chemists approved standard methods[24]. Total dietary fiber content (n = 4) was determined by enzymatic gravimetric method of Asp *et al.*[25]. The total carbohydrate content was determined by difference from the analysis of moisture, ash, crude fat and crude protein contents.

2.7. Statistical analysis

Results were expressed as mean ± SE. Data of each experiment were statistically analyzed using SAS version 6.12. One way ANOVA and the Duncan's multiple range test were used to determine the differences among treatment means. The Pearson's correlation coefficient was used for the correlation analysis. $P < 0.05$ was regarded as significant.

3. Results

3.1. Anti-diabetic related properties

3.1.1. In vitro starch digestion rate

In vitro starch digestion rate of selected varieties was given in Table 1. All the varieties showed significantly low *in vitro* starch digestion rate compared to white bread ($P < 0.05$). *In vitro* starch digestion rate of Dik Wee, Sudu Heeneti, Masuran and Goda Heeneti were 52.40 ± 1.44, 53.44 ± 0.35, 53.76 ± 1.19 and 62.84 ± 0.20 respectively when *in vitro* starch digestion of white bread was considered as 100. Statistically significant ($P < 0.05$) differences were observed among the varieties for *in vitro* starch digestion of selected rice varieties. Dik Wee, Sudu Heeneti and Masuran showed significantly low digestion rate compared to Goda Heeneti ($P < 0.05$).

Table 1

In vitro starch digestion rate of selected Sri Lankan traditional rice varieties.

Variety/Bread	Total sugars (mg/mL)			*In vitro* starch digestion rate
	1 h	2 h	3 h	
Dik Wee	0.13 ± 0.00	0.35 ± 0.02	0.52 ± 0.04	52.40 ± 1.44[a]
Sudu Heeneti	0.19 ± 0.01	0.46 ± 0.01	0.58 ± 0.01	53.44 ± 0.35[a]
Masuran	0.15 ± 0.01	0.39 ± 0.01	0.59 ± 0.02	53.76 ± 1.19[a]
Goda Heeneti	0.20 ± 0.00	0.45 ± 0.00	0.69 ± 0.00	62.84 ± 0.20[b]
White bread	0.33 ± 0.01	0.75 ± 0.02	1.09 ± 0.09	100

Data presented as mean ± SE (n = 4). Mean values of *in vitro* starch digestion rate with different letters are significantly different at $P < 0.05$.

3.1.2. MGO mediated protein glycation inhibitory activity

MGO mediated protein glycation inhibitory activity of rice bran extracts was given in Table 2. Bran extracts of all the varieties showed MGO mediated protein glycation inhibitory activity with significant differences ($P < 0.05$) among the varieties. Bran extract of Goda Heeneti and Dik Wee showed the highest inhibitory activity among the varieties studied. The order of potency of inhibitory activity among the selected varieties were Goda Heeneti = Dik Wee > Sudu Heenti > Masuran.

Table 2

MGO mediated protein glycation inhibitory activity. μg/mL.

Rice variety	Concentration					IC_{50}
	25	50	100	200	400	
Goda Heeneti	6.26 ± 1.58	21.74 ± 2.05	34.32 ± 2.34	51.82 ± 2.37	67.16 ± 2.05	174.77 ± 6.65[a]
Dik Wee	30.82 ± 1.41	35.98 ± 0.82	42.65 ± 0.86	54.78 ± 0.53	69.43 ± 0.26	187.26 ± 6.85[a]
Sudu Heeneti	5.69 ± 2.88	15.81 ± 1.76	18.16 ± 2.68	40.51 ± 0.33	59.99 ± 0.56	321.44 ± 13.72[b]
Masuran	7.05 ± 0.87	10.64 ± 1.15	22.88 ± 2.08	32.22 ± 2.77	52.86 ± 2.94	342.87 ± 0.43[c]

Data presented as mean ± SE (n = 3). IC_{50} values with different letters are significantly different at $P < 0.05$. IC_{50} of rutin: (63.36 ± 0.67) μg/mL.

Table 3

Acetyl-cholinesterase and butyryl-cholinesterase inhibitory activities of bran extracts of selected Sri Lankan traditional rice varieties. μg/mL.

Rice variety	Acetyl-cholinesterase inhibitory activity					Butyryl-cholinesterase inhibitory activity				
	Concentration				IC_{50}	Concentration				IC_{50}
	25	50	100	200		12.5	25	50	100	
Sudu Heeneti	44.36 ± 0.39	58.79 ± 0.45	66.55 ± 0.04	68.26 ± 0.55	37.00 ± 0.68a	39.84 ± 0.66	64.52 ± 1.40	79.80 ± 0.45	86.56 ± 0.57	18.50 ± 0.60a
Dik Wee	37.28 ± 1.27	47.86 ± 1.33	56.56 ± 1.42	63.15 ± 0.42	77.03 ± 2.37b	30.27 ± 3.29	49.18 ± 0.10	69.74 ± 0.51	81.36 ± 0.98	30.33 ± 0.32b
Masuran	27.75 ± 0.72	44.71 ± 3.77	52.16 ± 0.39	58.60 ± 0.18	88.27 ± 6.64c	25.11 ± 0.27	50.99 ± 1.99	65.75 ± 0.23	78.27 ± 1.16	30.20 ± 1.96b
Goda Heeneti	17.51 ± 0.39	26.09 ± 0.72	33.52 ± 0.39	39.30 ± 1.42	291.00 ± 3.54d	18.11 ± 2.37	32.57 ± 4.59	41.91 ± 0.50	50.68 ± 0.18	96.60 ± 0.56c

Data presented as mean ± SE (n = 3). IC_{50} values with different letters are significantly different at $P < 0.05$. IC_{50} of galanthamine, AChE: (0.46 ± 0.02) μg/mL; IC_{50} of galanthamine, BChE: (3.03 ± 0.01) μg/mL.

3.1.3. Acetyl and butyryl-cholinesterase inhibitory activity

Acetyl and butyryl-cholinesterase inhibitory activity of bran extracts of selected rice was given in Table 3. Bran extracts of all the varieties showed moderate acetyl and butyryl-cholinesterase enzyme inhibitory activities compared to the reference drug used in this study. Inhibition of butyryl-cholinesterase was more prominent compared to acetyl-cholinesterase inhibition. The differences observed among the varieties for both enzyme inhibitory activities were statistically significant ($P < 0.05$). Bran extract of Sudu Heeneti showed significantly high ($P < 0.05$) activity, while bran extract of Goda Heeneti showed the lowest ($P < 0.05$) inhibitory activity for both enzymes. The order of acetyl and butyryl-cholinesterase inhibitory activity of selected varieties were Sudu Heeneti > Dik Wee > Masuran > Goda Heeneti.

3.1.4. Anti-hyperglycemic activity

Anti-hyperglycemic activity of rice bran extract was given in Figure 1. The results revealed that rice bran of Masuran significantly reduced the glycemia levels in rats at 15 and 30 min after administration of rice bran extract at 1 g/kg body weight.

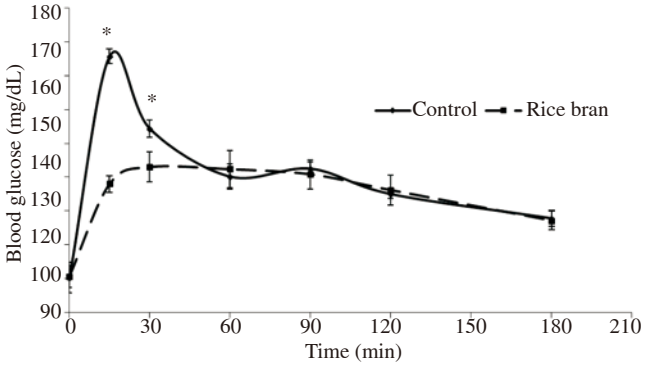

Figure 1. Blood glucose values obtained after the oral administration of starch (2 g/kg body weight) alone (Control) or with (1 g/kg body weight) rice bran extract.

Results expressed as mean ± SE (n = 8). *: $P < 0.05$ *vs.* Control at the same time point.

3.2. Physicochemical properties of the rice grain

Physical properties of rice varieties studied were presented in Table 4. Grain length and width of selected varieties ranged from (5.16 ± 0.05) to (5.98 ± 0.08) mm and (1.88 ± 0.04) to (2.48 ± 0.08) mm respectively. According to grain size classification used, Masuran, Dik Wee and Sudu Heeneti had medium grain size while Goda Heeneti was a short grain variety. Length to width ratio of selected varieties ranged from 2.10 ± 0.00 to 3.03 ± 0.00. Among the varieties studied, only Dik Wee had slender shape and other varieties had bold shape. Chemical properties of rice varieties tested were presented in Table 5. Significant differences ($P < 0.05$) were observed among the varieties for studied chemical properties except dietary fiber content of the rice grain. The moisture content varied between (10.90 ± 0.12)% to (11.70 ± 0.09)%. Ash and fat contents were ranged from (1.45 ± 0.08)% to (1.81 ± 0.04)% and (2.18 ± 0.10)% to (2.71 ± 0.35)% respectively. Among the varieties studied, Goda Heeneti had the highest ash content while Masuran had the highest fat content. The protein content was appreciably high (> 10%) for all tested varieties in this study. Specially, Goda Heeneti and Sudu Heeneti varieties showed the highest protein contents [(12.20 ± 0.02)% and (12.08 ± 0.03)% respectively]. The total carbohydrate content ranged from (83.69 ± 0.17)% to (85.22 ± 0.73)% respectively. The total dietary fiber content was between 4.68% and 5.15% and it was statistically insignificant ($P > 0.05$) among the varieties.

Table 4

Physical properties of selected Sri Lankan traditional rice varieties.

Rice variety	Color	Length (mm)	Grain size	Width (mm)	Length: width	Grain shape
Masuran	Red	5.98 ± 0.08	Medium	2.36 ± 0.05	2.53 ± 0.01	Bold
Dik Wee	Red	5.70 ± 0.07	Medium	1.88 ± 0.04	3.03 ± 0.00	Slender
Sudu Heeneti	Red	5.62 ± 0.13	Medium	2.48 ± 0.08	2.27 ± 0.00	Bold
Goda Heeneti	Red	5.16 ± 0.05	Short	2.46 ± 0.05	2.10 ± 0.00	Bold

Data presented as mean ± SE (n = 5). Short: Size classification < 5.5 mm; Medium: 5.5–6.3 mm. Bold: Length to width ratio 2.0–3.0; Slender: > 3.0.

Table 5

Chemical composition of selected Sri Lankan traditional rice varieties.

Rice variety	Protein	Fat	Ash	Total carbohydrate	Dietary fiber
Sudu Heeneti	12.08 ± 0.03^{a}	2.63 ± 0.17^{a}	1.60 ± 0.05^{ab}	83.69 ± 0.17^{b}	5.15 ± 0.07^{a}
Goda Heeneti	12.20 ± 0.02^{a}	2.18 ± 0.10^{a}	1.81 ± 0.04^{a}	83.81 ± 0.08^{b}	5.04 ± 0.16^{a}
Masuran	10.63 ± 0.63^{b}	2.71 ± 0.35^{a}	1.45 ± 0.08^{b}	85.22 ± 0.73^{a}	4.78 ± 0.11^{a}
Dik Wee	11.70 ± 0.08^{a}	2.39 ± 0.12^{a}	1.61 ± 0.18^{ab}	84.30 ± 0.18^{ab}	4.68 ± 0.41^{a}

Data presented as mean ± SE (n = 4 each). Mean values in a column with different letters are significantly different from $P < 0.05$. Crude protein, crude fat, ash, total carbohydrate and dietary fiber contents are given on % dry weight basis.

4. Discussion

Diabetes mellitus is a chronic metabolic disease increasing in epidemic proportions throughout the world including Sri Lanka[1,2,26]. Recent study has shown that Sri Lankan population currently has over 10% of diabetes prevalence. Further, prevalence of pre-diabetes is 11.5% and overall dysglycemia in the country

is 21.8%[26]. Therefore, dietary management is important for prevention and management of diabetes and its complications. As rice is the staple food in Sri Lanka, identification of low glycaemic rice varieties is necessary for glycaemic control in diabetes patients. Araya *et al.* showed a positive correlation of *in vitro* carbohydrate digestion rate and the glycaemic response in men and indicated that *in vitro* determination of carbohydrate digestion rate can be used as a simple and inexpensive method to estimate the biological response of high carbohydrate meals[27]. As such, we used *in vitro* starch digestion rate method to predict the glycaemic response *in vivo*. Dik Wee, Sudu Heeneti and Masuran varieties showed low starch digestion rate compared to white bread indicating that these varieties may be low glycaemic index rices. Many factors can influence the starch digestion rate, such as the nature of starch (amylose content), physical form, dietary fiber, protein and lipids, presence of antinutrients, enzyme inhibitors and food processing[15]. Our previous study has shown that brans of varieties used in this study had high anti-amylase activity[14]. Further, significant negative correlations were observed with fat content (r = -0.63; P < 0.05) and anti-amylase activity (r = -0.63; P < 0.05) to *in vitro* starch digestion rate possibly indicating its suitability in diabetic management strategies.

Anti-hyperglycaemic activity of rice bran was studied for the bran extract of Masuran variety since we have previously shown that it had the highest α-amylase inhibitory activity *in vitro*[14]. The results of this study clearly revealed that rice bran of Masuran possessed anti-hyperglycemic action. According to a recent study by Jung *et al.*, administration of phenolic acid fraction of the rice bran to diabetic mice significantly reduced the blood glucose level by elevating glucokinase activity and production of glycogen in the liver compared to the control group suggesting that phenolic acid fraction of the rice bran may be beneficial for treatment of type 2 diabetes[28]. We have shown that bran extract of Masuran had high polyphenolic content and it is positively correlated with the α-amylase inhibitory activity of the rice bran[14]. Therefore, anti-hyperglycaemic action of rice bran extract may be due to the presence of high polyphenolic content in the rice bran.

Prolonged hyperglycemia leads to glycation of proteins resulting in the formation of advanced glycation end products (AGEs) which are positively correlated with the development of diabetes complications[29]. The process of protein glycation is a series of complex reactions and only few compounds which play important roles in developing diabetes complications have been characterized to date[29]. One of this is MGO which is a highly reactive dicarbonyl compound[29]. Discovery of inhibitors which can trap or inhibit the MGO induced AGE formation would offer a potential therapeutic approach for the prevention of diabetes complications[29]. Bran extracts of Goda Heeneti and Dik Wee had significantly high MGO mediated protein glycation inhibition compared to the other varieties. The inhibitory activities against AGE formation of vitamin B1 and B6 derivatives such as pyridoxamine and thiamine pyrophosphate have mainly been attributed to their abilities to scavenge reactive carbonyl compounds[30]. Pigmented rice bran is a good natural source of vitamin B1 and B6 derivatives[31]. Therefore, vitamin B1 and B6 may be responsible, at least partly, for the observed MGO mediated protein glycation inhibitory activity. To the best of our knowledge, this is the first report of MGO mediated protein glycation inhibitory activity of rice bran from any variety of rice worldwide.

Type 2 diabetes mellitus is a risk factor for some neurological diseases including Alzheimer's disease[3]. In recent years, Alzheimer's disease has been even referred to as type 3 diabetes and hypothesized that it is caused by inadequate production of acetylcholine in the brain[3-5]. In this study, we have shown that the selected rice bran possesses marked AChE inhibitory activity. Therefore, consumption of these rice varieties with bran intact could increase the acetylcholine level and would be beneficial for management of Alzheimer's disease. There are several reports indicating that the reduction of AChE activity can be compensated by increasing BChE activity[32,33]. Rice bran of this study also had remarkable BChE inhibitory activity which could also contribute to enhancing the acetylcholine levels. Currently, BChE inhibitors such as cymserine analogues, and the dual inhibitor of both AChE and BChE such as rivastigmine are used therapeutically for treating Alzheimer's disease and related dementias[32]. In a recent *in vivo* study, it has shown marked AChE inhibitory activity from rice berry, variety of purple pigmented rice[34]. However, this is the first report of AChE inhibitory activity from rice bran of a Sri Lankan rice variety and BChE inhibitory activity from bran of any rice variety globally.

Physicochemical properties studied showed that selected varieties are red in color and grain size and shape were mostly medium and bold respectively. Further, all the selected varieties had considerably high protein content. Crude fat, dietary fiber and total carbohydrate contents are mostly within range of other rice varieties published to date[9,13]. Our previous studies showed that all the selected varieties in this study had high amylose content (> 25%) and desirable sensory (taste, aroma, appearance, cohesiveness, tenderness) and starch gelatinization properties[12]. Therefore, selected Sri Lankan traditional rice varieties in this study may have the potential to promote as physicochemically sound rices having range of anti-diabetic related properties for prevention and management of diabetes and its related complications.

Brans of all the selected Sri Lankan traditional rice varieties had MGO mediated protein glycation inhibition and acetyl and butyryl-cholinesterase inhibitory activities. This study also showed that brans of Masuran in rat model had anti-hyperglycemic activity and Sudu Heeneti, Masuran and Dik Wee could be considered as having low glycaemic indexes. Further, all the selected rice varieties had consumer preferred physicochemical properties. This is the first study to report MGO mediated protein glycation inhibitory activity and butyryl-cholinesterase inhibitory activity for any rice variety worldwide. Findings of this study indicate the possibility of promoting these selected Sri Lankan traditional red rice as a supplement for management of diabetes and its related complications.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Jain S, Saraf S. Type 2 diabetes mellitus-Its global prevalence and therapeutic strategies. *Diabetes Metab Syndr Clin Res Rev* 2010; **4**: 48-56.

[2] American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; **33**: S62- 9.

[3] Akter K, Lanza EA, Martin SA, Myronyuk N, Rua M, Raffa RB. Diabetes mellitus and Alzheimer's disease: shared pathology and treatment? *Br J Clin Pharmacol* 2011; **71**(3): 365-76.

[4] Talbot K, Wang HY, Kazi H, Han LY, Bakshi KP, Stucky A, et al. Demonstrated brain insulin resistance in Alzheimer's disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J Clin Invest* 2012; **122**(4): 1316-38.

[5] De Felice FG, Lourenco MV, Ferreira ST. How does brain insulin resistance develop in Alzheimer's disease? *Alzheimers Dement* 2014; **10**: S26-32.

[6] Padmavathi M. Chronic disease management with nutraceuticals. *Int J Pharm Sci Invent* 2013; **2**(4): 1-11.

[7] Bigliardi B, Galati F. Innovation trends in the food industry: the case of functional foods. *Trends Food Sci Tech* 2013; **31**: 118-29.

[8] Nagendra Prasad MN, Sanjay KR, Shravya Khatokar M, Vismaya MN, Nanjunda Swamy S. Health benefits of rice bran - A review. *J Nutr Food Sci* 2011; **1**: 108.

[9] Sompong R, Siebenhandl-Ehn S, Linsberger-Martin G, Berghofer E. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. *Food Chem* 2011; **124**: 132-40.

[10] Bhonsle SJ, Sellappan K. Grain quality evaluation and organoleptic analysis of aromatic rice varieties of Goa, India. *J Agric Sci* 2010; **2**(3): 99-107.

[11] Rajapakse RMT, Sandanayake CA, Pathinayake BD. Foot prints in rice variety improvement and its impact on rice production in Sri Lanka. In: Kotagama HB, Wijesekara GAW, Wijesundara DSA, editors. Proceedings of the Annual Symposium of the Department of Agriculture; 2000. Sri Lanka: Department of Agriculture.

[12] Food Technology Section. Properties of some traditional rice varieties of Sri Lanka. Sri Lanka: Industrial Technology Institute and the Department of Agriculture; 2011.

[13] Gunaratne A, Wu K, Li D, Bentota A, Corke H, Cai YZ. Antioxidant activity and nutritional quality of traditional red-grained rice varieties containing proanthocyanidins. *Food Chem* 2013; **138**: 1153-61.

[14] Premakumara GAS, Abeysekera WKS, Ratnasooriya WD, Chandrasekharan NV, Bentota AP. Antioxidant, anti-amylase and anti-glycation potential of brans of some Sri Lankan traditional and improved rice (*Oryza sativa* L.) varieties. *J Cereal Sci* 2013; **58**: 451-6.

[15] Thompson LU, Button CL, Jenkins DJA. Phytic acid and calcium affect the *in vitro* rate of navy bean starch digestion and blood glucose response in humans. *Am J Clin Nutr* 1987; **46**: 467-73.

[16] Lunceford N, Gugliucci A. Ilex paraguariensis extracts inhibit AGE formation more efficiently than green tea. *Fitoterapia* 2005; **76**: 419-27.

[17] Bhadra S, Mukherjee PK, Bandyopadhyay A. Cholinesterase inhibition activity of *Marsilea quadrifolia* Linn. an edible leafy vegetable from West Bengal, India. *Nat Prod Res* 2012; **26**: 1519-22.

[18] Institute for Laboratory Animal Research. *Guide for the care and use of laboratory animals*. 8th ed. Washington: The National Academic Press; 2011.

[19] Kasabri V, Afifi FU, Hamdan I. Evaluation of the acute antihyperglycemic effects of four selected indigenous plants from Jordan used in traditional medicine. *Pharm Biol* 2011; **49**(7): 687-95.

[20] Kasabri V, Afifi FU, Hamdan I. *In vitro* and *in vivo* acute antihyperglycemic effects of five selected indigenous plants from Jordan used in traditional medicine. *J Ethnopharmacol* 2011; **133**(2): 888-96.

[21] Tiwari AK, Swapna M, Ayesha SB, Zehra A, Agawane SB, Madhusudana K. Identification of proglycemic and antihyperglycemic activity in antioxidant rich fraction of some common food grains. *Int Food Res J* 2011; **18**(3): 915-23.

[22] NAPHIRE. Technical reference guide on grains postharvest operations. National Postharvest Institute for Research and Extension. Munoz, Nueva Ecija, Philippines; 1994.

[23] National Food Authority. Primer on Philippine grains standardization programme. Quezon City: National Food Authority; 2013. [Online] Available from: http://nfa.gov.ph/icon/files/issuances/pgspnew.pdf [Accessed on 20th June, 2015]

[24] AOAC. *Official methods of analysis*. 17th ed. Washington: Association of Official Analytical Chemists; 2000.

[25] Asp NG, Johansson CG, Hallmer H, Siljeström M. Rapid enzymatic assay of insoluble and soluble dietary fiber. *J Agric Food Chem* 1983; **31**: 476-82.

[26] Katulanda P, Ranasinghe P, Jayawardana R, Sheriff R, Matthews DR. Metabolic syndrome among Sri Lankan adults: prevalence, patterns and correlates. *Diabetol Metab Syndr* 2012; **4**: 24.

[27] Araya H, Contreras P, Alviña M, Vera G, Pak N. A comparison between an *in vitro* method to determine carbohydrate digestion rate and the glycemic response in young men. *Eur J Clin Nutr* 2002; **56**(8): 735-9.

[28] Jung EH, Kim SR, Hwang IK, Ha TY. Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/KsJ-db/db mice. *J Agric Food Chem* 2007; **55**(24): 9800-4.

[29] Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. *Korean J Physiol Pharmacol* 2014; **18**(1): 1-14.

[30] Neves D. Advanced glycation end-products: a common pathway in diabetes and age-related erectile dysfunction. *Free Radic Res* 2013; **47**: 49-69.

[31] Satter MA, Ara H, Jabin SA, Abedin N, Azad AK, Hossain A, et al. Nutritional composition and stabilization of local variety rice bran BRRI-28. *Int J Sci Technol* 2014: **3**(5): 306-13.

[32] Greig NH, Lahiri DK, Sambamurti K. Butyryl-cholinesterase: an important new target in Alzheimer's disease therapy. *Int Psychogeriatr* 2002; **14**: 77-91.

[33] Bajda M, Więckowska A, Hebda M, Guzior N, Sotriffer CA, Malawska B. Structure-based search for new inhibitors of cholinesterases. *Int J Mol Sci* 2013: **14**: 5608-32.

[34] Pannangrong W, Wattanathorn J, Muchimapura S, Tiamkao S, Tong-Un T. Purple rice berry is neuroprotective and enhances cognition in a rat model of Alzheimer's disease. *J Med Food* 2011; **14**(7-8): 688-94.