Indian Journal of Traditional Knowledge
Vol 20(3), July 2021, pp 707-715

Anti-hyperglycemic effect of bran extracts of two traditional rice varieties on alloxan-induced diabetic rats

Abubakar Sadiq Tanko[a,b] & Nazrim Marikkar[a,c,]*

[a]Department of Biochemistry, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor DE, Malaysia

[b]Department of Biochemistry, Faculty of Sciences, Bauchi State University Gadau, PMB 65, Bauchi, Nigeria

[c]National Institute of Fundamental Studies, Hanthana Road, Kandy, Sri Lanka

E-mail: nazrim.ma@nifs.ac.lk

Received 05 May 2020; revised 22 October 2020

Beras merah (BM) and *Beras hitam (BH)* are two traditional rice varieties popular among the tribal communities living in Ba'kelalan region of Sarawak, East Malaysia. They are grown in fertile paddy lands at 3000 feet above the sea level according to the traditional farming system. In this study, we attempted to investigate the impact of oral administration of bran extracts (RBE) of *B. merah* and *B. hitam* on Alloxan-induced diabetic rats. Four-week long treatment of animals with RBE of varying doses (400 and 200 mg/kg) were monitored using anti-hyperglycemic activity and several blood parameters. Changes in body weight and blood parameters, which include blood glucose, liver enzymes and blood proteins, were also assessed using relevant assays. Results showed that RBEs of both *B. merah* and *B. hitam* possessed good antidiabetic potentials. The group administered with 400 mg/kg of *B. merah* (BM 400) showed the highest antidiabetic efficacy compared to 200 mg/kg of the same extract (BM 200). However, RBE of *B. hitam* displayed anti-hyperglycemic activity only at moderate level. It can be concluded that traditional rice variety *B. merah* would be a good potent sources of natural anti-diabetic agents.

Keywords: Animal study, Anti-hyperglycemic activity, Blood glucose level, Diabetic management, Traditional rice bran
IPC Code: Int Cl.[21]: A01G 22/22, A23J 1/12, A61K 45/06, A61P 7/12

Diabetes mellitus is a metabolic disorder of carbohydrate metabolism characterized by chronic hyperglycemia, resulting in the inability of human pancreas to produce enough insulin or the cells poor response for insulin[1,2]. As at 2019, about 9.3% of the world adult population was reportedly living with diabetes and it has been predicted that this will rise to almost 11% by 2045[3]. Prevalence of diabetes among Malaysian adult population aging above 30 years has been increasing perpetually from 8.3 to 14.9 to 18.3% in 1996, 2006 and 2019, respectively[4,5]. According to Wan Nazaimon *et al.*[6], the overall prevalence of diabetes in the country was around 22.9% of the total population in 2013. Stress, sedentary lifestyle, increased obesity and unhealthy eating pattern are identified as contributing factors for increasing diabetes during the past two decades[7]. Failure to arrest the rising trend in diabetes in the country would negatively impact the health of the nation, social welfare of the people and economic status due to diabetic complications that might ensue.

Malaysia being an Asian country produces several hybrid rice varieties for white rice consumptions. For cooking purpose, most of the eateries and household use polished rice which gives an appealing white colour. Polishing helps remove the outer bran layer of the rice grain during milling of rice. According to a review by Sivamaurthi *et al.*[8], bran of rice is identified as one of the nutrient-rich by-products generated in abundance by the rice processing sector. It is generally believed that the brans of traditional pigmented rice are far better than those of commercial hybrid rice owing to occurrence of high amounts of tocopherols, tocotrienols, γ-oryzanol, unsaturated fatty acids etc[9]. Despite these merits, traditional rice consumption is still not popular among the main stream Malaysian public probably due to lack of promotional campaigns and scarcity of information related to their nutritional values. As a matter of fact, traditional rice varieties are considered to be toxic-free since they do not require excessive use of

*Corresponding author

fertilizer or pesticides due to drought tolerance and resistance for pest and diseases. As the chances of agrochemical exposure is minimal, traditional rice consumption can be a solution to address increasing cancer incidence among consumers. Research has provided ample evidence for the benefits of bioactive constituents of rice bran, which are attributed for multiple health claims on human subjects[8].

Malaysian rice germplasm consists of mainly hybrid rice along with several indigenous varieties namely, *Adan halus* (AH), *Adan kasar* (AK), *Salleh halus* (SH), *Salleh Kasar* (SK), *Beras merah* (BM), *Beras hitam* (BH) and *Nanung* (NNG)[10,11]. Some of these traditional rice varieties are confined to indigenous ethnic groups living in the Sarawak region of Malaysia where rice farming is done mainly using traditional, unmechanized methods. Conducive climate at 3000 feet above the sea level and fertile virgin soil in Ba'kelalan region of Sarawak provide many opportunities for the tribal community to cultivate and self-sustain with these traditional rice varieties. Low level of literacy among the tribal communities and remote location of Ba'kelalan region for public access had hindered previous scientific investigations on traditional rice varieties. According to a preliminary assessment undertaken recently, out of the seven different traditional rice varieties investigated, rice brans of *B. merah* and *B. hitam* were found to exhibit highest antioxidative and anti-hyperglycemic properties[10]. Nonetheless, further exploration of these findings using an animal-based *in vivo* study was not performed previously to confirm the above mentioned claim about RBEs on diabetic subjects. Hence, this study was aimed to investigate the anti-hyperglycemic potential of RBEs of *B. merah* and *B. hitam* on normoglycemic and alloxan-induced diabatic rats.

Materials and Methods

Rice bran sampling

Bran samples of two varieties of Malaysian traditional rice namely, *B. merah* (BM) *and B. hitam* (BH) were collected in triplicate from milling factories located at Ba'kelalan region of Sarawak, Malaysia. Samples sieved for extra-fine uniform particles of less than 325 mesh were heat-treated for 2 min at 2450 MHz using a convection oven and kept under refrigerated condition (4-5°C) until further analysis.

Experimental animals

For the experiment, healthy male Sprague Dawley rats (180-220 g) with no prior drug treatment were procured from Saintifik Enterprise Sdn Bhd, Selangor D. E., Malaysia. They were kept in ventilated cages and fed with a normal commercial pellet diet and water ad libitum for two weeks to acclimatize prior to the commencement of the study.

Ethical consideration

This study was performed in compliance to the Malaysian Guidelines for Good Clinical Practice. The prior approval for the protocol was granted by the Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (UPM) giving the registration number; Reg. No. UPM/IACUC/ AUP-R023/2015.

Preparation of RBEs

Ten grams of each sample, after being soaked for 24 h, was extracted with 70:30 ethanol: water (V/V) mixture at room temperature. The extract was then centrifuged with Eppendorf 8510R centrifuge (Eppendorf, Germany) at 8300 rpm speed for 10 min and filtered with Whatman # 1 filter paper. The solvent was subsequently evaporated under reduced-pressure using a Buchi Model R-205 rotary evaporator (Buchi, Switzerland). The semisolid extracts (RBEs) were frozen at -20 ± 1°C for 24 h and subsequently freeze-dried using Virtis bench-top pro freeze dryer SJIA-10N (FD-1B-50) (Virtis, New York) for 3 days until they were completely dried. Portions of each freeze-dried RBEs were dissolved in water to prepare different doses required to administer in the animals.

Study of anti-hyperglycemic activity of RBEs

Method described by Sangeetha *et al.*[12] was adopted with modification to assess the anti-hyperglycemic activity of the bran extracts in glucose loaded hyperglycemic animals. In brief, forty-eight animals fasted for 16 h were assigned randomly into 4 equal groups (n = 12/group). They were divided randomly into six treatment groups (n = 7) and administered with BM and BH extracts at different concentrations as described here: Group I: Normal control animals received vehicle only (10 mL/kg b.w); Group II: Animals treated with BH 400 mg/kg b.wt; Group III: Animals treated with BH 200 mg/kg b.wt; Group IV: Animals treated with BM 400 mg/kg b.wt; Group V: Animals treated with BM 200 mg/kg b.wt.); Group VI: Animals treated with Glibenclamide (10 mg/kg b.wt.). After 30 min of oral dosing, the animals were fed with glucose (2 g/kg b.w.) orally using gastric intubation. Samples of blood

were withdrawn from the tail at 0, 30, 60 and 120 min of glucose administration. Blood Glucose Level (BGL) were estimated by the glucose oxidase enzymatic method using a commercial Accu-check active glucometer and test-strips. Water was used as a vehicle since all samples were soluble in water.

Study of anti-diabetic activity of RBEs

Diabetes was induced with a single intraperitoneal injection of 100 mg/kg of alloxan monohydrate to the over-night fasted (8 h) animals. Animals were divided randomly into 7 groups having five animals each (n = 5) based on treatments given. BGL of the induced animals were measured with Accu-check active glucometer from tail pinch 72 h after alloxanization; animals with fasting BGL of 200 mg/dL and above were identified as diabetic. The selected animals were randomly divided into seven treatment groups (n = 7) and administered the treatments as described here: Group I: Positive control group of diabetic animals treated with 5 mg/kg of Glibenclamide; Group II: Negative control group of non treated diabetic animals; Group III: Normal control group of non diabetic animals; Group IV: Diabetic animals treated with 400 mg/kg *B. merah* alcoholic extract; Group V: Diabetic animals treated with 200 mg/kg *B. merah* alcoholic extract; Group VI: Diabetic animals treated with 400 mg/kg *B. hitam* alcoholic extract; Group VII: Diabetic animals treated with 200 mg/kg *B. hitam* alcoholic extract. Body weights and blood glucose levels of the animals were measured at three (3) days interval until the final day (28^{th} Day) of the studies. On the final day of the experiment, the animals fasted-overnight were sacrificed by terminal bleeding through cardiac puncture after anaesthesia injection.

Determination of blood biochemical parameters

After the period of experimentation, the animals fasted-overnight were sacrificed under anaesthesia. Blood samples were withdrawn through cardiac puncture with the aid of sterile syringes and needles and dispensed into sterile tubes. The blood was allowed to clot for few hours after which it was subjected to centrifugation at 3, 500 rpm for 30 min in order to separate the serum from the whole blood. Serum was removed with sterile needles and syringes and then stored in a freezer until further analysis. Estimation of other biochemical parameters namely, Alanine amino transferase (ALT), Triglycerides (TAG), total protein (TP) and Albumin/globulin (ALB) were carried out by standard enzymatic methods using Hitachi 902 automated biochem analyser.

Statistical analysis

All measurements of the experiment were carried out in triplicate data (n = 3) and the data were presented in mean ± standard deviation (SD). Results were statistically analysed using one-way analysis of variance (ANOVA) with IBM SPSS software package (version 21.0). When F values were significant, mean differences were compared using Duncan's multiple range test at the 5% level of probability.

Results

Anti-hyper glycemic activities of RBEs

Results of normo-glycemic studies conducted on rats with intact pancreas are shown in Table 1. Before administering the extracts, no significant ($p>0.05$) difference was noticed among all groups with regard to their fasting BGL. After 30 min of administration of 2 g/kg glucose, there was a significant ($p<0.05$) rise in the BGL of all groups. Moreover, significant ($p<0.05$) differences in BGL were noticed after 120 min of administration among different groups treated with different extracts. The group treated with the known antidiabetic drug, glibenclamide showed the lowest BGL, which was significantly ($p<0.05$) lower than those of any other group. Interestingly, BGL of all groups treated with different doses of RBE extracts were found to be significantly ($p<0.05$)

Table 1 — Effect of rice bran extracts of BH and BM on BGL (mg/dL) of glucose-loaded hyperglycemic rats

Time (Min)	BM400	BM200	BH400	BH200	GBLMD	NEG
0	87.8 ± 9.2^{a}	85.8 ± 6.7^{a}	82.2 ± 7.3^{a}	86.2 ± 5.0^{a}	84.0 ± 3.1^{b}	88.6 ± 4.4^{a}
30	119.8 ± 8.3^{b}	116.8 ± 7.3^{b}	108.8 ± 8.9^{a}	146.4 ± 6.1^{c}	109.4 ± 8.1^{a}	121.2 ± 4.2^{b}
60	92.6 ± 6.1^{ba}	102.8 ± 6.5^{c}	118.6 ± 2.7^{d}	126.80 ± 3.6^{e}	84.4 ± 4.2^{a}	118.2 ± 7.3^{c}
90	95.6 ± 8.5^{b}	92.6 ± 2.1^{b}	107.4 ± 4.6^{c}	113.8 ± 5.0^{d}	50.6 ± 2.7^{a}	107.0 ± 7.7^{c}
120	80.6 ± 4.4^{b}	82.8 ± 5.6^{b}	89.8 ± 5.8^{b}	88.4 ± 9.1^{b}	42.6 ± 4.6^{a}	101.0 ± 7.5^{c}

The values represent Mean ± Standard deviation triplicate analysis. Means within each raw bearing different superscript are significantly ($p<0.05$) different. Abbreviations: BGL: blood glucose level; NEG: Negative control administered with water, BM400: Group treated with 400 mg/kg of *Beras merah*, BM200: Group treated with 200 mg/kg of *Beras merah*, BH400: Group treated with 400 mg/kg of *Beras hitam*, GBLMD: Group treated with 5 mg/kg of glibenclamide.

lower than that of the negative control group. In addition, the group treated with BM400 mg/kg extract exhibited the lowest BGL when compared to any other RBE treated group. This showed that RBE of *B. merah* was dose dependent and dose level of 400 mg/kg displaying the highest hypoglycemic activity. The fall in BGL seen among RBE treated groups could be due to insulin mimetic activities exhibited by the RBEs which facilitated the fast absorption of glucose into the tissues and organs requiring them.

Anti-diabetic activities of RBEs

Data presented in Table 2 shows the results of changes in fasting BGL among different diabetic groups induced with single injection of alloxan monohydrate. Prior to inducing animals with alloxan, the fasting BGL of the rats in all groups were within the normal physiological range. After three days of the injection, rats in all induced groups exhibited hyperglycemia; their fasting BGL were significantly ($p<0.05$) higher than that of non alloxanized group. After confirmation of diabetic status of the rats, treatment continued until the 28th day of the study (last day). All diabetic groups treated with different doses of the RBEs (BH400, BM400, BH200 and BM200) responded differently throughout the course of the treatments. According to the BGL of the last day of this experiment, none of the diabetic treated groups had their BGL normalized to those of the non alloxanized group. Although there was a gradual decline in the BGL of all treated groups, their values were significantly ($p<0.05$) higher than that of the non-treated normal control group. In fact, the results shown by RBE of *B. merah* is encouraging as it was dose dependent with the dose of 400 mg/kg showing the highest hypoglycemic activity.

Changes in body weight

Changes in body weights of Alloxan-induced diabetic group and the normal control group before and after treatments are compared as shown in Table 3. Prior to the induction of diabetes, animals in all groups did not show any significant ($p<0.05$) difference with regard to their body weights. Remarkable decreases in body weight were observed

Table 2 — Changes in fasting BGL (mg/dL) of different groups of diabetic rats

Day	N.A.	GBLMD	NEG	BH400	BH200	BM400	BM200
1	78 ± 2.9^{b}	79.6 ± 2.1^{ab}	81.0 ± 2.4^{ab}	79.8 ± 6.2^{ab}	81.8 ± 2.4^{ab}	82.4 ± 1.3^{a}	80.6 ± 2.4^{ab}
4	78.8 ± 4.1^{d}	223.2 ± 24.1^{bc}	232.6 ± 19.4^{bc}	210.6 ± 11.6^{c}	244.6 ± 16.2^{b}	269.2 ± 30.1^{a}	222.8 ± 2.9^{bc}
7	77.0 ± 8.6^{d}	132.4 ± 30.7^{c}	224.6 ± 6.2^{b}	224.2 ± 5.1^{b}	252.4 ± 15.5^{a}	256.2 ± 28.8^{a}	228.2 ± 5.4^{b}
10	71.8 ± 13.1^{e}	114.4 ± 15.1^{d}	217.2 ± 4.2^{bc}	232.6 ± 11.2^{ab}	241.0 ± 21.8^{a}	238.2 ± 20.8^{a}	211.4 ± 6.8^{c}
13	79.4 ± 9.0^{e}	108.0 ± 8.1^{d}	225.8 ± 5.8^{ab}	225.6 ± 5.77^{ab}	231.2 ± 12.8^{a}	214.6 ± 17.7^{bc}	202.8 ± 15.5^{c}
16	78.2 ± 6.1^{f}	108.8 ± 8.2^{e}	232.8 ± 5.9^{a}	226.2 ± 3.3^{a}	205.6 ± 13.0^{b}	191.6 ± 9.3^{c}	173.05 ± 19.2^{d}
19	70.2 ± 6.1^{e}	98.0 ± 12.4^{d}	235.6 ± 7.1^{a}	197.8 ± 12.0^{b}	196.4 ± 21.0^{b}	181.0 ± 13.0^{b}	146.2 ± 15.3^{c}
22	83.0 ± 7.5^{f}	102.6 ± 11.8^{e}	240.4 ± 6.5^{a}	185.6 ± 16.8^{b}	194.4 ± 17.7^{b}	165.6 ± 10.4^{c}	136.4 ± 13.2^{d}
25	73.2 ± 10.6^{g}	89.4 ± 6.0^{f}	243.0 ± 8.8^{a}	162.8 ± 8.2^{c}	183.0 ± 8.2^{b}	143.6 ± 14^{d}	128.4 ± 7.5^{e}
28	79.4 ± 11.4^{f}	94.2 ± 12.7^{e}	246.2 ± 4.2^{a}	149.4 ± 7.1^{c}	184.6 ± 8.7^{b}	117.0 ± 5.2^{d}	125.4 ± 7.1^{d}

Data are presented as Mean ± Standard deviation. Means within rows with different superscripts are significantly different (at $p<0.05$). Abbreviations: BGL: blood glucose level; BM400: Group treated with 400 mg/kg of *Beras merah*, BH400: Group treated with 400 mg/kg of *Beras hitam*, BH200: Group treated with 200 mg/kg of *Beras hitam*, BH200: Group treated with 200 mg/kg of *Beras hitam*, GBLMD: Group treated with 5mg/kg of glibenclamide, NEG: Negative control administered with water; N.A. Normal control.

Table 3 — Changes in body weights (g) of different groups of rats starting from 1st until 28th day of the study

	1st Day - Body weight (g)	28th Day - Body weight (g)	% Change in body weight
Normal control	215.4 ± 13.4^{a}	275.4 ± 8.4^{b}	+27.85
Diabetic control	212.60 ± 16.55^{a}	169.00 ± 21.21^{a}	-20.51
Diabetic+ BH (400 mg/Kg b.wt.)	210.29 ± 14.9^{a}	192.00 ± 5.66^{c}	-8.70
Diabetic+ BH (200 mg/Kg b.wt.)	212.43 ± 15.00^{a}	199.25 ± 29.94^{c}	-6.20
Diabetic+ BM (400 mg/Kg b.wt.)	222.40 ± 11.2^{a}	209.80 ± 28.55^{c}	-5.67
Diabetic+ BM (200 mg/Kg b.wt.)	209.70 ± 14.7^{a}	195.75 ± 37.21^{c}	-6.65
Diabetic+ Glibenclamide (5 mg/Kg b.wt.)	220.55 ± 11.2^{a}	217.00 ± 32.01^{c}	-1.61

Data are presented as Mean ± Standard deviation. Mean values with different superscripts within the column are significantly different ($p<0.05$). Abbreviations: BM400: Group treated with 400 mg/kg of *Beras merah*, BH400: Group treated with 400 mg/kg of *Beras hitam*, BH200: Group treated with 200 mg/kg of *Beras hitam*, BH200: Group treated with 200 mg/kg of *Beras hitam*, GBLMD: Group treated with 5 mg/kg of glibenclamide, NEG: Negative control administered with distilled-water.

Table 4 — Effect of different treatments on biochemical parameters of Alloxan induced diabetic rats on the 28th day of treatment

Parameters	N.A.	GBLMD	NEG	BH400	BH200	BM400	BM200
ALT (U/L)	36.5 ± 9.2^{e}	54.7 ± 3.0^{d}	94.2 ± 14.9^{a}	74.4 ± 18.8^{bc}	83.2 ± 7.9^{ab}	56.3 ± 9.6^{cd}	78.3 ± 24.2^{ab}
TAG (mg l/dL)	14.2 ± 2.6^{i}	15.0 ± 1.3^{hi}	31.5 ± 3.3^{f}	19.0 ± 2.7^{gh}	20.2 ± 6.9^{g}	15.7 ± 1.5^{hi}	18.7 ± 0.3^{gh}
T.P. (g/L)	77.7 ± 3.4^{jk}	79.7 ± 3.8^{jk}	81.1 ± 3.0^{jk}	76.7 ± 6.1^{k}	83.0 ± 4.4^{j}	79.9 ± 4.4^{jk}	76.8 ± 4.3^{k}
ALB (g/L)	$36.8 \pm .9^{lm}$	38.8 ± 2.4^{lm}	39.7 ± 1.2^{l}	34.8 ± 6.9^{lm}	38.6 ± 2.2^{lm}	35.3 ± 3.5^{lm}	34.3 ± 5.9^{lm}

Each value presented as Mean ± Standard deviation. Mean values with different superscripts in the same row are significantly different ($p<0.05$). Abbreviations: BM400: Group treated with 400 mg/kg of *Beras merah;* BH400: Group treated with 400 mg/kg of *Beras hitam;* BM200: Group treated with 200 mg/kg of *Beras merah;* BH200: Group treated with 200 mg/kg of *Beras hitam;* GBLMD: Group treated with 5 mg/kg of glibenclamide; NEG: Negative control administered with distilled water; ALT: Alanine amino transferase; ALB: Albumin, TAG: Triglycerides, T.P.: Total protein.

among Alloxan-induced diabetic groups when compared to the normal control group, which exhibited a significant ($p<0.05$) increase by 27.85%.

Biochemical parameters

Liver enzymes

The results presented in Table 4 compare the effects of different treatment of RBEs on biochemical parameters namely alanine amino transferase (ALT), total protein (T.P), albumin (ALB) and triglycerides (TAG) of alloxan induced diabetic rats. ALT is a liver specific enzyme whose levels in blood exceeding the normal range will be indicative of hepatic necrosis. According to results, alloxan induced-diabetic groups had displayed significantly ($p<0.05$) higher alanine amino transferase levels when compared to the non alloxanized groups. However, between the positive control group and the non alloxanized group, there was no significant ($p>0.05$) difference. This showed that the positive controlled group treated with glibenclamide had their ALT levels lowered close to the non alloxanized groups. Although the BM400 exhibited a significantly higher enzyme levels than the non alloxanized group, there was no significant ($p>0.05$) difference between the BM400 and the positive controlled group with regard to their ALT levels. This shows that the concentration of BM at 400 mg/kg is found to be almost as potent as glibenclamide at 5 mg/kg. The other two groups namely BH200 and BM200 showed no significant difference in ALT levels with the negative control group indicating a low potency of anti-diabetic activities.

Triglycerides

According to results presented in Table 4, the control group (NEG) displayed significantly ($p<0.05$) higher levels of triglycerides when compared to any other treated group. All other treated groups also had significantly ($p<0.05$) higher triglycerides levels when compared to the normal group except groups treated with glibenclamide and BM400. However, the remaining groups namely, BH400, BH200 and BM200 were able to show potentials of lowering of high blood triglycerides. These results further confirmed that the rise in BGL of alloxan-induced diabetic animals was accompanied by a rise in serum triglyceride levels. The abilities of the RBEs to reduce diabetic hypertriglyceridemia could be due to their potentials of controlling hyperglycemia.

Total blood protein and albumin

Results of the total protein and albumin of blood samples from different groups are shown in Table 4. Among the different groups, there was hardly any significant ($p>0.05$) difference with regard to total protein and albumin content.

Discussion

Anti-hyperglycemic properties

Addressing diabetes in the early stage of incidence is always important to prevent long-term complications leading to severe damages to body's systems including blood vessels and nerves[13]. Making an effort to maintain the blood glucose level within the normal range would help prevent persistence of hyperglycemia and minimize complications linked to diabetes[14]. One important approach in the management of diabetes is to control the postprandial BGL by inhibiting the activities of the carbohydrate hydrolysing enzymes in the small intestine through drugs. To date, several synthetic drugs have been used as inhibitory agents as they possess the ability to bind to the active sites of the enzymes to inhibit their catalytic activities thereby causing a decrease in the release of glucose from carbohydrate digestion[15]. As alternatives for synthetic anti-diabetic drugs, which have been implicated for many side effects, natural anti-diabetic agents especially from traditional medicinal or dietary plants have been explored by

several research groups[2]. Liyanagamage *et al.*[16] recently compiled the studies performed to investigate the uses of traditional medicinal plants for glycemic control among diabetic subjects. According to the traditional system of medicine such as Ayurveda and Siddha, use of extracts of medicinal plants is found to be effective for diabetic control. Apart from taking medicinal extracts, diabetic patients are advised to consume traditional food or drinks such as chyme prepared with *Osbeckia octandra*, curries prepared with *Lassia spinos*, salads prepared with *Centella asiatica*, spices of *Trigonella foenum-graecum*, fruits of *Phyllanthus embelica* and drink of *Camellia sinesis* as a part of their diet[17]. In place of wheat flour, food products based on millet, legumes and fenugreek seeds are considered to be beneficial as diabetic diet. Consumption of fenugreek seeds, for instance, as a supplement on a daily basis has been shown to provide antidiabetic effect among prediabetes. Recently, Gaddam *et al.*[18] reported that the dietary intake of 10 g fenugreek seeds/day in prediabetes subjects could help to lower the rate of conversion into diabetes. Among the different types of Sri Lankan rice, traditional varieties such as *Goda Heeneti*, *Sudu Heeneti*, *Dik Wee* and *Masuran* are found to be good sources of essential minerals, vitamins, amino acids, insoluble fibre and coloured pigments[19]. As such, they were reported to display lower glycemic index (GI), which is beneficial to maintain glycemic control among diabetic subjects[20]. This observation was reaffirmed through another study conducted using the traditional rice varieties of Malaysia, where the RBEs of *B. merah* and *B. hitam* exhibited inhibitory effects on carbohydrate hydrolyzing enzymes, which implied their potency to be used in diabetic management[10]. In the application side, Godakumbura *et al.*[21] suggested that use of traditional rice grain with its bran in herbal porridge would make the product more nutritious and healthier.

Animal models are frequently employed as tools to assess the therapeutic potential of novel anti-diabetic agents. Although several synthetic drugs are employed to induce diabetes in animals, Alloxan monohydrate and Streptozotocin have become most prominent among researchers due to their efficacy[22]. In our study, Alloxan monohydrate was chosen as diabetogenic drug due to its low-cost. According to Makheswari & Sunderam[23], a single injection of alloxan at a dose of 140 – 180 mg/kg (5% w/v), usually 150 mg/kg intraperitoneally is sufficient to induce diabetes in rats after fasting of at least eight (8) hours. When administered to animals like mice, rats, rabbits and dogs, it accumulates in the pancreatic beta cells and is subsequently taken up via the GLUT2 glucose transporter[24]. Alloxan is believed to cause excess production of reactive oxygen species leading to cytotoxicity in pancreatic beta cells, which reduces the synthesis and release of insulin[25]. As a result, increase in BGL will occur owing to lack of absorption of glucose by the cells, which would force the cells to utilize fatty acids and amino acids as energy source, thereby leading to the reduction of fat and proteins in the body[26]. Naito *et al.*[27] further stated that the body weight losses in diabetic animals can also happen due to non-enzymatic glycosylation prompted by hyperglycemia since non-enzymatic glycosylation is one of the processes that lead to depreciation of body protein.

Normo-glycaemic studies of our investigation aimed at seeing the efficacy of RBEs to counter the increasing BGL after a glucose load without actually tempering with the pancreatic islets[28]. At thirty minutes after glucose administration, the BGL peaked rapidly from fasting BGL value, but then subsequently decreased. Rapidity in postprandial hyperglycemia is a normal physiological process in the body, but its persistence would be deleterious. Both RBEs exhibited considerable potential to bring down the BGL remarkably after 120 min of administration. Antioxidants present in the RBEs could be possibly helping to normalize the rising blood glucose level through inhibition of alpha amylase. This is in conformity with the findings reported by Yao *et al.*[29] and Premakumara *et al.*[20] who previously discussed the inhibitory potentials of rice bran on carbohydrate digestive enzymes.

In the present study, all diabetic groups exhibited hyperglycemia after three days of inducing alloxan. The spike in fasting BGL observed among diabetic groups would be probably due to damages in the islets of Langerhans in the pancreas caused by administering alloxan monohydrate[30]. However, gradual decline in the levels of blood glucose among all treated groups was observed due to the effect of RBEs especially RBE of *B. merah.* According to the preliminary results of our investigation, RBEs of both *B. merah* and *B. hitam* were found to be potent in inhibiting carbohydrate hydrolyzing enzymes; this was attributed to bioactive compounds occurring in pigmented rice[9]. Yao *et al.*[29] previously found that

anthocyanin contents of coloured grains are higher than those of the white grains, which might help in the inhibition of enzymes hydrolyzing carbohydrates. According to another study by Manoharan *et al.*[31], oral administration of ethanolic extract of plant *Pippali* (*Piper longum* L.) restored the normal BGL in diabetic rats within 45 days, which indicated that the extract stimulated the normal homeostasis of blood glucose in diabetes.

Blood parameters generally serve as important biomarkers in determining the function or impairment of certain body organs. After studying the hepato-renal protective and anti-diabetic effects of ziyuglycoside II methyl ester in Type 2 diabetic mice, Son *et al.*[28] stated that the rise in ALT levels of the mice was due to hepatic necrosis which could exacerbated by diabetes progression. However, Navarro *et al.*[32] argued that the increase in the activities of the enzymes was mainly due to outflow of the enzymes caused by leakage from the cytosol of the liver into the blood stream as a results of hepatic necrosis which might have resulted from alloxan toxicity. In our study, administration of RBEs to the individual treatment groups indicated a decline in the enzyme activities towards normalcy. Yousef & El-naga[33] previously made similar observations while studying the effect of garlic oil on the levels of various enzymes in the serum. The abilities of the RBEs especially BM400 to lower the levels of the liver enzymes was likely due to antioxidant activities of the extracts which prevented further generation of the hepatic cells by formation of free radicals through alloxan induced peroxidation. The ability displayed by *B. merah* at 400 mg/kg showed it as a potential anti-diabetic agent to prevent or curb diabetic complication.

Diabetes is reported to have a strong nexus with impaired metabolism of lipids, which could cause an increase in the levels of blood lipids putting diabetes patients at risk of complications like atherosclerosis[34]. According to Barrett & Udani[35], insulin stimulates the uptake of blood glucose into cells of the skeletal muscle and liver where they are stored in the form of glycogen. It is also responsible for increase in fatty acid synthesis for storage purpose. However, decrease in insulin sensitivity or resistance was said to cause deposition of lipids in liver and skeletal muscle thereby increasing insulin resistance which exacerbate the chances of developing or worsening Type-2 diabetes and heart disease. A decrease in the amounts of blood protein is usually caused in conjugation with glucose and protein peroxidation, both of which progress during the state of chronic hyperglycemia and oxidative stress. This phenomenon is quite prevalent especially among diabetic patients. Moreover, both processes that lead to fall in protein usually take a longer period of time before beginning to expose[12,36]. In the present study, maintenance of protein to near normal level could be due to the effect of RBEs on blood glucose levels that lead to the non-progression of processes like protein oxidation and glycation, both of which can lead to a decline in blood protein in the state of chronic hyperglycemia[26]. It could also be partly due to the shorter time period of this study, which is less than 3 months. This might not give the actual protein levels as affected due to the processes like glycation, which would normally last for at least three (3) months before indication to be seen in the protein levels.

Conclusions

Brans of the two traditional rice varieties of this study are highly useful as nutraceutical as well as functional food because the oral administration of the RBEs of them was found to be effective in gradual reduction of BGL in Alloxan induced diabetic rats. This is in conformity with the preliminary results obtained for α-amylase and α-glucosidase assays of RBEs of these two rice types. Out of the two traditional rice bran types investigated, *B. merah* was found to emerge as a more potent anti-hyperglycemic agent. Although weight losses were common among most of the Alloxan-induced animal groups, restoration of their blood biochemical parameters to near normal levels was attained after treatment with RBEs especially *B. merah*. The overall findings suggest that rice from these two varieties with their bran can become base material for functional foods such as herbal rice porridges, rice noodles, rice crackers etc. If active compounds responsible for the antidiabetic and antioxidative activities of these two traditional rice brans are discovered, they can be isolated and used as active ingredients for pharmaceutical purposes or drug development.

Acknowledgement/Funding Source

The authors acknowledge the financial support from a fundamental research grant (FRGS/2/2013/SG01/UPM/02/5) awarded by Ministry of Higher Education Malaysia. The authors also acknowledge the Padiberas Nasional Berhad (BERNAS) for providing samples of commercial rice bran.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contributions

Abubakar Sadiq Tanko performed the experiment and drafted the manuscript; Nazrim Marikkar designed the study, supervised the work and edited the manuscript.

References

1 Nair S, Kavrekar V & Mishra A, In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts, *Eur J Exp Biol*, 3 (2013) 128–132.

2 Sales P M, Souza P M, Simeoni L A & Silveira D, α-Amylase inhibitors: A review of raw material and isolated compounds from plant source, *J Pharm Pharm Sci*, 15 (2012) 141–83.

3 International Diabetes Federation, I., *Diabetes Atlas* International Diabetes Atlas, ed. Sixth. 2014, www.diabetesatlas.org: Karakas Print. 136.

4 Shahar S, Kee CC, Ab. Rahman, Jamalludin, Mohamad Nor, *et al*. The third national health and morbidity survey: Prevalence of obesity, and abdominal obesity among the Malaysian elderly population, *Asia-Pac J Public Health*, 24 (2012) 318–329.

5 National Health and Morbidity Survey of Malaysia (NHMS) 2019. Accessed at https://codeblue.galencentre.org/ 2020/05/29.

6 Wan Nazaimoon W M, Md Isa S H, Wan Mohamad W B, Khir A S, Kamaruddin N A, *et al*., Prevalence of diabetes in Malaysia and usefulness of HbA1c as a diagnostic criterion, *Diabetic Med*, 30 (2013) 825–28.

7 Jarald E, Joshi S B & Jain D C, Biochemical study on the hypoglycaemic effects of extract and fraction of *Acacia catechu* willd in alloxan-induced diabetic rats, *Int J Diabet Metabol*, 17 (2009) 63–69.

8 Sivamaruthi B S, Kesika P & Chaiyasut C, A comprehensive review on anti-diabetic property of rice bran, *Asian Pac J Trop Biomed*, 8 (2018) 79–84.

9 Jun H I, Song G S, Yang E I, Youn Y & Kim Y S, Antioxidant activities and phenolic compounds of pigmented rice bran extracts, *J Food Sci*, 77 (2012) C759–C764.

10 Tanko A S, Marikkar J M N, Salleh A, Azrina A & Jivan M, Evaluation of brans of different rice varieties for their anti-oxidative and anti-hyperglycemic potentials, *J Food Biochem*, 41 (2017) e12295.

11 Barakatun Nisak M Y, Ruzita A T & Norimah A K, Glycaemic index of eight types of commercial rice in Malaysia, *Mal J Nutr*, 11 (2005) 151–163.

12 Sangeetha A, Prasath Sriram G & Subramanian S, Antihyperglycemic and antioxidant potentilas of *Sesbania grandiflora* leaves studied in STZ induced experimental diabetic rats, *Int J Pharm Sci Res*, 5 (2014) 2266–2275.

13 Sharma R, Dave V, Sharma S, Jain P & Yadav S, Experimental models on diabetes : A comprehensive review, *Int J Adv Pharm Sci*, 4 (2013) 1–8.

14 Kumar S, Kumar V, Rana M & Kumar D, Enzymes inhibitors from plants: An alternate approach to treat diabetes, *Pharmacogn Comm*, 2 (2012), 18–33.

15 Kanahaiya L A & Kumar J A, Alpha amylase inhibitor formulation development using cowpea - A novel entities, *Int J Pharm Stud Res*, 1 (2010) 64–71.

16 Liyanagamage D S N K, Jayasinghe S, Attanayake, A P & Karunaratne V, Medicinal plants in management of diabetes mellites: A review, *Cey J Sci*, 49 (2020) 03–11.

17 Ediriweera E R H S S & Ratnasooriya W D, A review on herbs used in treatment of diabetes mellitus by Sri Lankan Ayurvedic and traditional physicians, *AYU* 30 (4) (2009) 373–391.

18 Gaddam A, Galla C, Thummisetti S, Marikanty R K, Palanisamy U D & Rao P V, Role of fenugreek in the prevention of Type 2 diabetes mellitus in prediabetes, *J Diab Metab Dis*, (2015) 14:74

19 Abeysekera W K S M, Premakumara G A S, Ratnasooriya W D, Chandrasekharan N V, & Bentota, A P, Physicochemical and nutritional properties of twenty-three traditional rice (*Oryza sativa* L.) varieties of Sri Lanka, *J Coast Life Med*, 5(2017) 343-349.

20 Premakumara G A S, Abeysekera W K S M, Ratnasooriya W D, Chandrasekharan N V & Bentota A P, Antioxidant, anti-amylase and anti-glycation potential of brans of some Sri Lankan traditional and improved rice (*Oryza sativa L.*) varieties, *J Cereal Sci*, 58 (2013): 451–456.

21 Godakumbura, P, Prashantha, M A B & Thushara, N, Medicinal values of herbal poriddges, *Sri Lan Sci*, 5 (2019) 11–13.

22 Viana G S, Medeiros A C, Lacerda A M, Leal L K, Vale T G, *et al.*, Hypoglycemic and anti-lipemic effects of the aqueous extract from *Cissus sicyoides*, *BMC Pharmaco*, 8 (2004) 4–9.

23 Makheswari U & Sudarsanam D, A review on bio informatics for diabetic mellitus, *Int J Pharm Sci Res*, 3 (2012) 389–95.

24 Szkudelski T, The mechanism of Alloxan and Streptozotocin action in β Cells of the rat pancreas, *Physiol Res*, 50 (2001) 537–546.

25 King A J F, The use of animal models in diabetes research, *Brit J Pharmacol*, 166 (2012) 877–894.

26 Ojo R J, Segilola L I, Ogundele O M, Akintayo C O & Seriki S, Biochemical evaluation of lima beans (*Phaseolus lunatus*) in Alloxan induced diabetic rats, *ARPN J Agric Biol Sci*, 8 (2013) 302–309.

27 Naito Y, Nakanishi M, Suehiro A, Mukai J & Uchida K, Biological role and measurement of advanced glycation end products and their precursors, *Supplement*, 51 (2012) M-121.

28 Son D, Hwang S, Kim M, Park U & Kim B, Anti-diabetic and hepato-renal protective effects of Ziyuglycoside II Methyl ester in Type 2 diabetic mice, *Nutrients*, 7 (2015) 5469–83.

29 Yao Y, Sang W, Zhou M & Ren G, Antioxidant and alpha-glucosidase inhibitory activity of colored grains in China, *J Agric Food Chem*, 58 (2010) 770–774.

30 Yasodamma N & Alekhya C, Alloxan induced diabetic Albino rats, *Int J Pharm Pharm Sci*, 5 (2013) 577–583.

31 Manoharan S, Silvan S, Vasudevan K & Balakrishnan S, Antihyperglycaemic and antilipidoperoxidative effects of Piper longum dried fruits in alloxan induced Diabetic rats, *J Biol Sci*, 7 (2007) 161–168.

32 Navarro C M, Montilla P M, Martin A, Jimenez J & Utrilla P M, Free radicals scavenger and antihepatotoxic activity of rosmarinus, *Plant Med*, 59 (1993) 312–314.

33 Yousef M I & El-naga N I A, Biochemical study on the hypoglycemic effects of onion and garlic in Alloxan-induced diabetic rats, *Food Chem Toxicol*, 43 (2005) 57–63.

34 Uttra K M, Devrajani B R, Zulfiquar S & Shah A, Lipid profile of patients with diabetes mellitus (A multidisciplinary study), *World App Sci J*, 12 (2011) 1382–1384.

35 Barrett M L & Udani J K, A proprietary alpha-amylase inhibitor from white bean (*Phaseolus vulgaris*): A review of clinical studies on weight loss and glycemic control, *Nutri J*, 10 (2011) 24.

36 Stangl V, Dreger, H, Stangl K & Lorenz M, Molecular targets of tea polyphenols in the cardiovascular system, *Cardiovas Res*, 73 (2007) 348–358.