Micronutrients and phytochemicals content in various rice (*Oryza sativa* Linn.) samples control carbohydrate digestion variedly and present differential antioxidant activities: an *in vitro* appraisal

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High starch and carbohydrate content in rice (*Oryza sativa* Linn.) disparages this food responsible for postprandial hyperglycemic excursions. However, rice is an Asian food synonym. Additionally, in traditional Indian healing practices, rice is used for treatment of various disorders. In this research, we analyzed micronutrient and phytochemical contents in different rice samples available in market. Effect of aqueous-ethanol extract of each type of rice was evaluated against intestinal α-glucosidase to examine extract's influence on carbohydrate digestion. Free-radicals scavenging activities as a measure of antioxidant potential in different rice were also examined. Brown-rice presented highest (54%) α-glucosidase inhibition followed by parboiled-rice (52%), idly-rice (48%), hand-pounded rice (42%), dosa-rice (40%) and basmati-rice (39%). Polished white sona-masoori rice presented least enzyme inhibitory (31%) activity. Presence of higher α-glucosidase inhibitory activity was regarded as slow digesting rice that would impart lesser postprandial glycemic excursion. Brown and parboiled-rice presented highest ABTS$^{•+}$ radical scavenging (74%) activity whereas; idly-rice displayed highest DPPH scavenging (50%) activity. Higher polyphenol and niacin contents in rice were found significantly (p<0.0001 and p<0.01 respectively) correlated with enzyme inhibitory activity. Higher polyphenol content was found responsible for enhanced free-radicals scavenging activity. Increasing concentrations of niacin and pantothenic acid correlated with ABTS$^{•+}$ radical scavenging activity.

Keywords: Antioxidant activity, Carbohydrate digestion, α-Glucosidase inhibition, Micronutrients, Phytochemicals, Rice
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Rice (*Oryza sativa* Linn.), the grain of life; is food synonym for Asians[1]. Recently, in an international study presented at Glasgow UK, in European Congress on Obesity, it was disclosed that eating Japanese- or Asian-style diet, based on rice prevents people become obese[2]. Apart from its use as food, since ancient times, rice has been part of traditional medicine throughout India. In Ayurvedic texts, it is mentioned as *Śāli* (transplanted rice), *Vrihi* (broad casted rice) and *Sashtika* (summer rice that matures in 60 days; in Sanskrit language *sashtika* means sixty)[1,3]. Varieties of rice *viz. Rajanam, Krishna Sali, Red Samba, Munda Sali, Mahasali, Sugandha Sali, Thriya Sali, White Samba, Aalcha, Karhani, Kalimoonch, Maharaji, Bhajari, Dhanwar, Mehar, Saraiphol, Kari bhatta, Karikagga, Atikaya, Mullarya, Nivara, Erumakkari, Katheri, Kaflaya, Matali, Lal Dhan* have been known in different parts of India and were used against various ailments[1].

The local rice varieties such as *Atire, Kayame, Hallinga, Halaga, Nattijaddu, Peetasale, Pingara, Hallaga, Rajakayame, Chare, Kalame, Maskat, Bilehagga, Atikaraya, Gandasale, Mesebattha, Kajajaya, Gulavadi sanna, Nerambade, Kavalakannu, Kalakedodru, Moradda, Suggikayame, Atikaya, Katamunda, Kandalakutti, Chikkasale, Giresale, Karikagga, Bilikagga, Jerigesanna, Jadagi, Halaga-1, Hanasu, Kiruvannu* have been found beneficial in many disease conditions and are used traditionally by local people of coastal Karnataka state of India[4].

Similarly, wide genetic diversity among rice varieties representing *Śāli* (cultivated during monsoon season), *Boro* (cultivated preferably during winter season), *Jum* (adapted to dry land) and glutinous (commonly cultivated throughout the region as a source of grain for breakfast and dessert for many ethnic communities) have been reported to be cultivated in North-Eastern part of India[4]. Varieties such as *Lahi, Local Basmati, Borjahinga, Joha, Hati Hali, Balam, Lallatoi, Arfa, Mulahail, Guaroi,*

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Harinarayan, and *Bherapawa* are considered *Sali* rice. The *Boro* type rice constitue *Aubalam, Bashful* and *Moircha* whereas, *Papue, Sorpuna, Kawanglaawang* and *Mimutim* as *Jum* type rice and *Til Bora, Kaki beroin, Bas beroin, Barua beroin, Ranga borah* are cultivated as glutinous rice in the region[5].

Rice water or its gruel has been prescribed in Ayurveda to be taken with Ayurvedic formulations namely Kamdudha ras, Amritanav ras, Pradraripu ras, Mahagandhak ras, Swarn malati ras, Sutsekhar ras, Dughdavati, Pradak nasak churna, Laghumai ras, Pushpnag churna, Mukta sukti, and Sangrahat bhasm in order to either improve the efficacy of drugs or minimize the unwanted effects of such Ayurvedic preparations[1]. Traditional healers and farmer were acquainted with the healing power of rice preparations. Extract of brown rice have been used by the people as energy drink, increase lactation in child bearing mothers and nutritious drink to child, treatment of chronic gastric complications and jaundice. The sticky glutinous rice was found helpful in soothing stomach upset, nausea, heart burn and, brown rice extract has been reported beneficial in breast and stomach cancer[6]. According to Charak Samhita, consumption of red sali rice with old sashtika rice relieves fever (रक्तशाल्यादयः शस्ताः पुराणाः षष्टिकैः सह Ch.Chi 3/178)[7], and in case of chest, pelvis and head pain during that condition, the consumption of liquid gruel of red sali rice has been advised accordingly (पेयां वा रक्त शालीनां पार्श्वबस्तिशिरोरुजि Ch.Chi.3/181)[7]. Furthermore, consumption of sali and sashtika rice along with butter, ghee, goat's meat and, fresh wine has been proposed to check bleeding from hemorrhoids as per the shloka नवनीतघृतं छागं मांसं च सषष्टिकः शालिः । तरुणश्च सुरामण्डस्तरुणी च सुरा निहन्त्यस्रम् Ch.Chi.14.211)[7]. Similarly, regular consumption of sali rice along with barley (सेवित शोफाभिहितांश्च योगान् पाण्डवामयी शालीयवांश्च नित्यम्, Su.Su.44.37)[8] has been prescribed to relieve conditions like inflammatory swellings, anemia and vomiting and intake warm gruel soup of old sali rice along with roasted meat of wild animals (जीर्णशाल्योदनं स्निग्धमल्पमुष्णं द्रवोत्तरम् । भुञ्जानो जाङ्गलैर्मासैः शीघ्रं व्रणमपोहति (Su.Su.19.32) has been stipulated to promote wound healing according to Sushruta Samhita[8].

Unfortunately however, in spite of the presence of these wide varieties of rice in localized regions, scientific literatures[1,4] and their medicinal properties[3,6-9], the common consumer of modern day is hardly acquainted with the presence of such varieties of rice or even aware of their medicinal properties. Such information of varieties and the knowledge about medicinal values of rice is vanishing along with folks who lived life traditional ways. The newer generation rather is made scared about consumption of rice for the fact that it has high glycemic value. Industrial processing and polishing of rice in order to make grains whiter, smoother and silky attracts consumer's attention and easy cooking of such polished white rice made it preferable in almost every home kitchen. Processing and polishing in fact, removes vital micronutrients, vitamins and minerals from the grain and leaves grain with concentrated starch/carbohydrate. Higher consumption of such polished rice induces higher glycemic response and is associated with increased risk of type 2 diabetes especially in Asian population[10]. It is reported that consumption of each serving per day of white rice is responsible to increase about eleven percent the risk of type 2 diabetes development in this population[10]. On the other hand, the unpolished rice retains majority of its micronutrients and phytochemicals present in the bran layer and hence, appears red or brown in color. It is this rice that has been described healthy in Ayurvedic literatures beneficial and advocated by traditional healers[7,8].

The rate of digestion and glycemic response of a dietary material depends on several factors including starch/carbohydrate content and number of other micronutrients and phytochemicals that interfere with their digestion process. Elevated postprandial glycemic condition induces oxidative stress which is an independent risk factor aggravating endothelial dysfunction and development of diabetic vascular complications[11]. Therefore, rice which digests slowly and presents potent antioxidant activity may become preferred grain for consumption to avoid higher postprandial glycemic response, mitigate postprandial oxidative burden and development of diabetic cardiovascular complications.

In this research, we assessed *in vitro* the carbohydrate digestion resisting potential and free-radicals scavenging antioxidant activity in aqueous-ethanol extract of various type of rice available to the consumers in super markets. Simultaneously, efforts were also made to find correlation between the analyzed phytochemicals and micronutrients that influence carbohydrate digestion and antioxidant activities.

Material and Methods
Sample collection and preparation
Ready to cook rice samples were procured from super market with available specifications over the

counter. Aromatic, long grain 12 months old Basmati (BA) rice was of Haryana State origin. This rice after cooking are non sticky and spreads it's peculiar Basmati aroma. Par boiled (BO) rice after cooking gives non sticky and off white texture. This rice was 6 months old and originated from Aarani, Gingee Tiruvannamalai (Tamilnadu State). Twelve months old unpolished brown (BR) rice takes time for cooking and appears sticky. This rice originated from Miryalaguda, Kurnool (Telangana and Andhra Pradesh). Six months old Dosa (DO) rice was of Warangal (Telangana) origin. Grains of DO rice were uniform, coarse and give soft texture to dosa (a south India's popular dish). Kanchipuram, Tindivanam (Tamilnadu) originated, 6 months old, bold grain Idly (ID) rice gives puffy texture to Idlies when cooked. Nine months old medium size, uniform and elongated grains of hand pounded (HP) rice was of Nizamabad, Miryalguda (Telangana) origin. This rice looks very much similar to brown rice. Polished (PO) silky shining white rice is called Sona Masoori rice. It was 9 months old and originated from Nizamabad, Miryalguda (Telangana). This rice gives white and little sticky texture after cooking. It is very popular among customers. Pictures of rice samples are presented in Figure 1.

Five grams of each finely powdered rice samples were soaked in 50 mL of aqueous ethanol (1:1) solution for 24 hrs. Supernatant was filtered over Whatman filter paper. The fresh and clear aqueous-ethanol rice solution (AERS) was used for analysis of enzyme inhibitory and free radicals scavenging activities and, estimation of total polyphenol and flavonoids.

α- Glucosidase inhibition assay

Rat intestinal acetone powder was used as a source of crude intestinal α-glucosidase. In a 96 well micro plate, extract of each rice sample (20 µL AERS)) and 100 µL phosphate buffer (100 mM, pH 6.8) were incubated with 50 µL crude intestinal alpha glucosidase for 10 min before 50 µL of disaccharide substrate (5mM *p*-nitrophenyl α-D-glucopyranoside) was added. Rate of release of *p*-nitrophenol as a measure of disaccharide digestion was read at different time intervals (0-11 min) at 405 nm spectrophotometrically (BioTek[synergy4] multimode micro plate reader)[12]. Percentage of enzyme inhibition was calculated as $(A_c - A_t / A_c) \times 100$ Where, A_c represents the absorbance of control and A_t represents the absorbance of test sample.

Estimation of carbohydrates

Individual rice flours (0.5g) were mixed with 1 mL of water. Mixtures were heated at 37 °C for ten min. Cooled mixtures were centrifuged at 2000 rpm for 10 min at room temperature. Carbohydrates in supernatant (100 µL) were dehydrated with acidic-anthrone reagent (400 µL; 0.2% anthrone in concentrated H_2SO_4) for 5 min in 1.5 mL Eppendorf tubes. 100 µL of blue-green color furfural-anthrone complex were placed in 96-well micro plate and measured spectrophotometrically at 620 nm[13]. Serial dilutions of glucose solution (1 mg/mL) was prepared and reacted with anthrone reagent in same manner. The amount of carbohydrates in each rice sample was determined by using glucose-calibration curve. Results were expressed as mg/100g.

Estimation of starch content

Rice flours (50 mg) were incubated with hot (50 °C) ethanol (80%) for 5 min over hotplate. Mixtures were homogenized and centrifuged at 2000 rpm for 2 min at room temperature. Supernatants were discarded and residues were dried. Each residue was mixed with 65 µL perchloric acid ($HClO_4$) and 50 µL water, centrifuged at 2000 rpm for 2 min at room temperature. Suitable volume of anthrone reagent (0.2% in conc. H_2SO_4) was added to the each dried
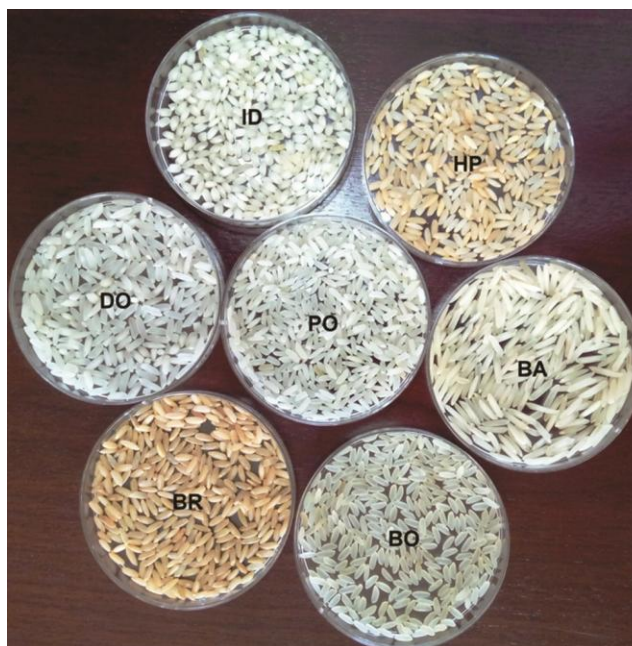

Fig. 1 — Photograph of different rice samples. BA-Basmati rice, BO-parboiled rice, BR-Brown rice, DO- this rice is sold as Dosa rice, ID- this rice is sold as Idly rice, HP-available in the market as hand pounded rice and PO-shining polished Sona Masoori Rice and is commonly recognized as polished rice.

residue and heated for 8 min and then cooled to room temperature. In a 96-well micro plate, 100 µL of clear cooled reaction mixture of each rice sample was placed and absorbance of blue-green color furfural-anthrone complex was measured spectro photometrically at 630 nm[14]. The amount of starch in each rice sample was determined by using glucose-calibration curve and expressed as mg/100g.

Estimation of total polyphenolic content

About 25 µL AERS of each rice sample was mixed with 2.5 mL distilled water and 250 µL Folin-Ciocalteu reagent and Na_2CO_3 (250 µL, 20%) in test tubes. The mixtures were thoroughly shaken for mixing and allowed to stand for 60 min at room temperature. 100 µL of each samples were placed in 96-well micro plate and absorbance was recorded at 765 nm spectrophotometrically[12]. Gallic acid was taken as standard and the content was measured in the samples were expressed as µg of GAE/mL.

Estimation of total flavonoid content

Briefly, in 96-well plate, 125 µL of AERS were mixed with 125 µL of 2% $AlCl_3.6H_2O$ and absorbance was read at 430 nm spectrophotometrically[12]. Rutin was taken as the standard drug and the flavonoid content in the samples were expressed in terms of Rutin Equivalent (µg RE/mL).

Antioxidant activity

ABTS+ radical cation scavenging

10 µL AERS of each rice sample were mixed with 190 µL of ABTS+ radical cation solution. The kinetics of ABTS+ radical cation decolorization was recorded at the intervals of 1 min for 30 mins spectrophotometrically at 734 nm[15]. The percentage of inhibition was calculated as (A_c-A_t/ A_c) x100 Where, A_c represents the absorbance of control and A_t represent the absorbance of test sample. Results were measured in triplicates.

DPPH radical scavenging

The reaction mixture contained 25 µL AERS of each rice sample , 125 µL of 0.1 M tris-HCl buffer (7.4) and 125 µL of 0.5 mM DPPH solution. The kinetics of DPPH radical decolorization was recorded at the interval of 1 min for 15 mins at 517 nm spectrophotometrically[15]. The percentage of inhibition was calculated as (A_c-A_t/ A_c) x100 Where, A_c represents the absorbance of control and A_t represent the absorbance of test sample.

Multicomponent micronutrients analysis

Chemicals and reagents

The analytical standards of biotin, folic acid, hesperetin, niacin, pantothenic acid, quercetin, riboflavin along with other chemical reagents and solvents of mass grade (methanol, acetonitrile, ethanol, ammonium formate, formic acid, sodium hydroxide and orthophosphoric acid) were procured from Sigma Aldrich. Ultrapure water obtained after filtration with 0.2 µ filter (Millipore, Synergy® 3 France) was used throughout the study.

Preparation of Standard Solution

The stock solutions of analytical standards were prepared by dissolving an amount of 10 mg of individual nutrients in a 10 ml of suitable solvent and stored in dark at 4°C. The mixture of nutrients was prepared by combining individual stock solutions to form a working standard of 0.1 mg/mL. Water soluble vitamins were dissolved in water. Flavonoids were dissolved in acetonitrile and quercetin was dissolved in methanol.

Instrumentation

Analysis was performed on ultra performance liquid chromatography-triple quadrupole mass spectrometer (UPLC-ESI-MS/MS) contain an acquity H-class UPLC and Xevo TQ-S micro MS system (Waters India Pvt Ltd., Milford). The UPLC consisted of a degasser, pump and detector with ESI source. Instrument was operated by Masslynx software version 4.1. The vortex mixer (Neuation, iSwix VT), ultrasonic bath (BANDELIN), centrifugation (HERMIE-Z300), weighing balance (Sartorius BSA224-CW), pH meter (Cole Parmer). HLB cartridges (Supelco super-select HLB 60 mg/3ml) used for clean-up of samples by solid-phase extraction were purchased from Sigma Aldrich (MO, USA). The solid-phase extraction with 12 position vacuum manifold for cleanup was used (Phenomenex). The water was purified with 0.2 µ PVDF filter (Millipore, Synergy® 3 France).

Analytical procedure

The extraction and analysis of selected nutrients were analyzed using our recently reported method[16]. Briefly, the powder sample (100 mg) placed in 15 mL centrifuge tube was extracted with 5.20 mL of mobile phase A (20 mM ammonium formate, 0.1% formic acid in water) and 3 mL of mobile phase B (20 mM ammonium formate, 0.1% formic acid in methanol) and vortexed for 3 min then sonicated for 5 min

followed by centrifugation for 5 min at 6,000 rpm. The resultant solutions were maintained at pH 5 followed by cleanup with solid-phase extraction system using supel-select SPE tubes (60 mg/3 ml). The SPE after loading and washing was eluted with 80% methanol. The eluent was injected into LC-MS/MS system for further analysis. The column used for analysis is BEH C_{18} column (100 mm $\times$ 2.1 mm, 1.7 µm particle size) in both, positive and negative modes with a source temperature of 150°C, capillary voltage at 3 KV and desolvation gas flow of 650 L/hr. The analytes (multi-class nutrients) were quantified in multiple reactions monitoring (MRM) mode. Optimal conditions for all the analytes are summarized in Table 1. Mobile phase A & B run in gradient program consisted of mobile phase A kept at 95% for 0.1-4 min, at flow 0.350 ml/min; then 4-8 min, 30% A flow at 0.300 ml/min; 8-10 min, 0% A flow at 0.400 ml/min; 10-13 min, 95% A flow at 0.350 mL/min with an injection volume of 5 µl. Samples were quantified with external standard method with a calibration range of 10-1000 ng/g with regression range of 0.997-0.999. Values obtained so were converted into µg/100g accordingly.

Statistical analysis

One-way ANOVA followed by Tukey's multiple comparison tests, and Unpaired-t test with Welch's correction was applied suitably to analyze data. Analyses were performed using Graph pad Prism 5.03.

Results and Discussion

Intestinal α-glucosidase is ultimate carbohydrate digesting enzyme present in intestinal brush borders that breaks disaccharides into monosaccharides *viz.* glucose and fructose. Therefore, the rate of food digestion by α-glucosidase is one of the primary factor governing incursions of glucose into the blood. Figure 2(a) presents influence of different rice

samples' extract on kinetics of intestinal α-glucosidase and bar graph in Figure 2(b) shows end-point values of decrease in enzyme activity potential by extracts. Steeper is the slope faster is the digestion of carbohydrate (Fig. 2a). It is evident from figure 2(a) that extract of BR rice followed by BO, ID and HP rice slowed down enzyme activity more potently than extract of other rice samples. Extract from PO rice presented least decline in enzyme activity (Fig. 2a). It was interesting to record that enzyme action started soon after addition of disaccharide substrate *p*-nitrophenyl α-D-glucopyranoside where α-glucosidase was preconditioned with PO and BA rice extract. Whereas, it took more than four minutes in the case of BR rice extract preconditioned enzyme followed by BO and ID extract preconditioning (Fig. 2a).

Figure 2(b) presents decrease in intestinal α-glucosidase activity at 11th minute (end-point time) under influence of different rice extracts. The decline in α-glucosidase activity due to priming with extract of BA (39%), DO (40%) and HP (42%) rice samples was found comparable as they did not differ significantly among each other (Fig.1b). Similarly, the fall in enzyme activity by BO (52%) rice extract was also found closer to BR (54%) and ID (48%) rice. However, it was significantly higher ($p<0.05$) with BR rice extract priming (54%) when compared with ID (48%) rice (Fig. 2b). Preconditioning of enzyme with PO rice extract poorly affected enzyme activity (31%) among studied rice samples (Fig. 2b). These results demonstrate that BR, BO and ID rice contain active principles that can appreciably slow down activity of carbohydrate digesting enzyme and hence consumption of these rice may impart lesser postprandial glycemic excursion than other rice like PO, BA, DO and HP.

For, rice is the prominent source of carbohydrate; it is first choice of food grains. The carbohydrate is a general term used as macronutrients content in food

Table 1 — Optimal conditions for the mass parameters for individual analytes

Analytes	MRM Transition-1 (m/z)	MRM Transition-2 (m/z)	Cone /CE (eV)	Dwell time (sec)
Niacin	124.00 > 78.00	124.00 > 80.00	30/18	0.012
Pantothenic acid	220.20 > 72.00	220.20 > 90.00	30/12	0.011
Biotin	245.00 > 97.00	245.00 > 227.80	20/11	0.011
Quercetin	303.10 > 153.00	-	-	0.011
Hesperetin	303.30 > 153.00	303.30 > 177.00	60/13	0.011
Riboflavin	377.20 > 172.00	377.20 > 243.00	30/19	0.011
Folic acid	442.00 > 176.00	442.00 > 295.00	50/20	0.01

CV= Cone voltage; CE= Collision energy

grain. Starch is a form of complex carbohydrate in food grains. The viscosity present in rice due to starch determines the quality of rice[17] and viscous nature of starch present in rice makes food more appealing and tastier[18]. Our analysis shows that starch content in BA and DO rice sample was significantly ($p < 0.001$ and $p < 0.009$ respectively) higher than carbohydrate content present in these two rice samples (Fig. 3) whereas carbohydrate content in ID rice was significantly ($p < 0.007$) higher than starch content (Fig. 3). Amongst seven collected samples from market, DO rice was found richest source of starch and carbohydrate (Fig. 3). Starch and carbohydrate content in studied rice samples were determined in whole rice grain. Although these parameters cannot be directly read and corroborated with their influence on enzyme activity, we made an attempt of finding correlation between starch/carbohydrate content in

rice samples with enzyme activity. Here, we subtracted the decreased enzyme activity values with hundred percent activity of the enzyme from figure 1b. Applying non-parametric correlation test (Spearman correlation), it was found that high starch and carbohydrate content in rice was significantly and positively ($p < 0.04$) correlated with the digestion of rice (Fig. 4). It is also important to note here that DO rice despite being richest source of starch and carbohydrate in our sampling (Fig. 3), decreased α-glucosidase activity better (40%) than PO rice (31%) (Fig. 2b). Similarly, the carbohydrate and starch content in ID and PO rice presented closer values (Fig. 3), decline in enzyme activity by ID rice extract however, was found 17% more than that present in PO rice (Fig. 2b). Analogous results were found for other rice samples also where starch and carbohydrate content was in close vicinity (Fig. 3) but
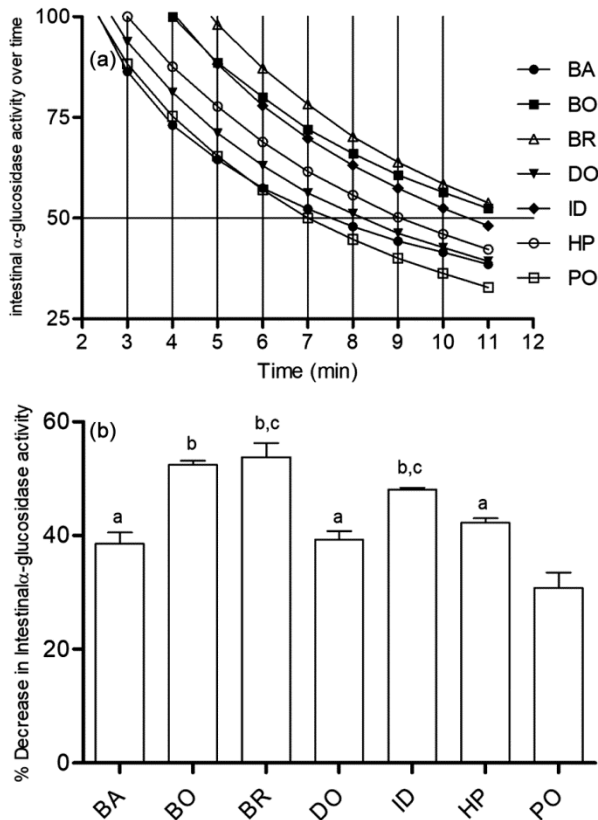

Fig. 2 — Rat intestinal α-glucosidase activity over time (a) under influence of different rice extracts and (b) % decrease in enzyme activity due to different rice extracts. Bars bearing letters 'a' and 'b' are not significantly different among each other however, 'c' has significant ($p < 0.05$) difference. Values represent mean±SD of three triplicate experiments in case of figure 'b' while mean value was plotted in figure 'a'. One-way ANOVA followed by Tukey's multiple comparison tests was applied to find differences within the bars.
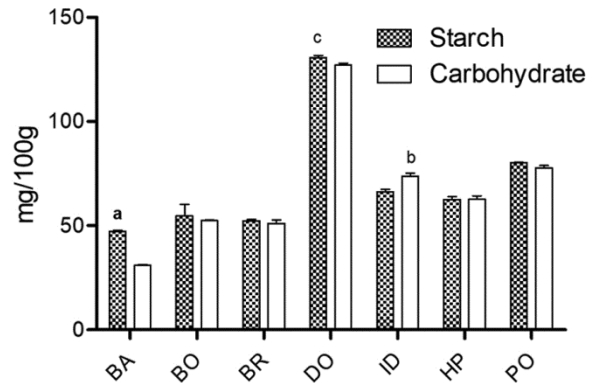

Fig. 3 — Starch and carbohydrate content in different rice samples. Values are mean±SD, n=3. Unpaired-t test with Welch's correction was applied to find difference between starch and carbohydrate content in individual rice. [a]$p < 0.001$, [b]$p < 0.0076$ and [c]$p, 0.0096$.
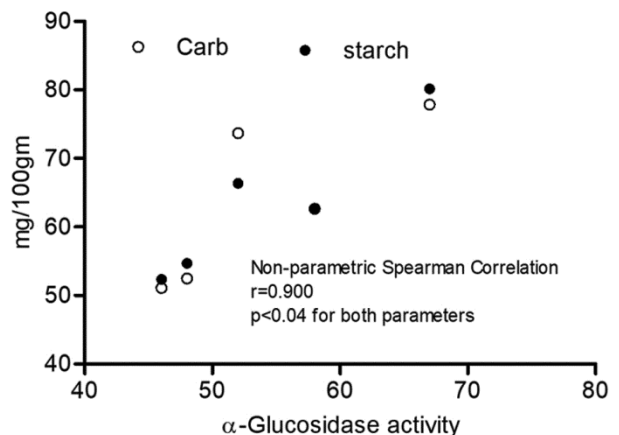

Fig. 4 — Correlation between carbohydrate and starch content in rice and intestinal α-glucosidase activity.

their influence on slowing down the enzyme activity was different (Fig. 2b). These observations disclose that besides starch and carbohydrates, there are other constituents in rice which determine their rate and fate of digestion.

Polyphenol-rich functional foods/nutraceuticals are emerging as newer food supplements to control postprandial hyperglycemic excursions in type 2 diabetics by virtue of their abilities to slow down activity of carbohydrate digesting enzymes[19]. Similarly, natural flavonoids have also been implicated in influencing activity of number of enzymes including intestinal α-glucosidase, responsible for multitude of therapeutic activities[20]. Examination of polyphenol and flavonoids contents in our study (Table 2) showed that there was great variation in polyphenol content in studied rice samples. The PO rice sample presented least polyphenol content where as BO followed by BR and ID rice displayed higher polyphenol content among studied rice samples (Table 2). Although, the total flavonoids content was recorded to be present in each rice sample, the differences were not much wider as observed for polyphenol content except in DO rice extract where flavonoids content was lowest (Table 2). It was found that polyphenol content in rice extract significantly (p<0.0001) affected activity of intestinal α-glucosidase (Fig. 5). More was the polyphenol content in rice sample; greater was the decline in intestinal α-glucosidase activity (Fig.5) and hence, slower the rate of digestion. Our analysis could not find correlation however, with flavonoids content in rice impinging enzyme activity. The flavonoids hesperetin and quercetin are well reported potent α-glucosidase inhibitors[19,21], and were found present in our sampled rice extract (Table 2). Because their concentration did not differ appreciably among the extract of studied rice samples (Table 2), their correlation with enzyme inhibitory could not be found in our analysis.

Our study finds that concentration of polyphenols in rice grain negatively influence rate of rice digestion and hence polyphenol-rich rice may become ideal substitute for better control of postprandial glycemic excursion after their consumption. It is important to mention here that polyphenol is a generic term used to classify compounds containing phenolic hydroxyl. The effect of polyphenolic compounds influencing α-glucosidase activity depends on their structural features. There are reports which show that polyphenols behave as double-edged sword and can also augment α-glucosidase activity[21,22]. The observation in our analysis that polyphenols present in rice extract slow down activity of intestinal α-glucosidase activity, warrants identification of α-glucosidase inhibitory polyphenols in different rice varieties. Identification of such compounds and their concentrations may help categories rice for their apt marketing and consumer education.
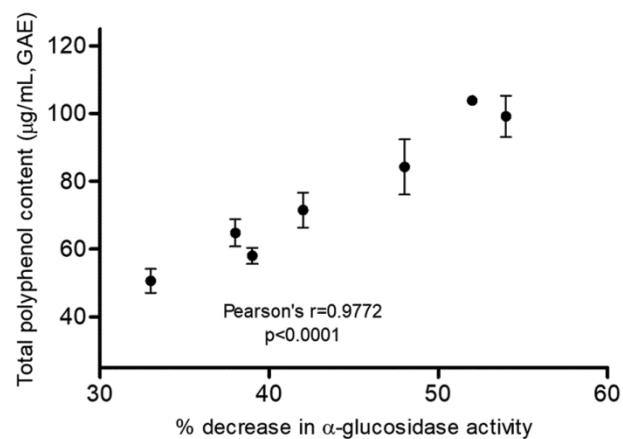

Fig. 5 — Influence of total polyphenol content in aqueous-ethanol extract in rice on intestinal α-glucosidase activity. Values represent mean±SD, n=3.

Table 2 — Phytochemical and micronutrient content in different market samples of rice.

	Total Polyphenol (µg/mL, GAE)	Total Flavonoids (µg /mL, RE)	Multicomponent micronutrients (µg/100g)						
			Niacin (Vit. B$_3$)	Pantothenic acid (Vit. B$_5$)	Biotin (Vit. B$_7$)	Riboflavin (Vit. B$_2$)	Folic acid (Vit. B$_9$)	Quercetin	Hesperetin
BA	64.8±0.004	875.36±0.04	58.6±0.74	3.4±0.12	35.5±0.86	19.9±0.57	2.0±0.87	0.8±0.20	0.8±0.44
BO	103.2±0.001	811.59±0.01	74.6±1.30	4.7±0.28	34.0±0.38	22.5±0.09	2.3±0.11	0.7±0.13	0.8±0.17
BR	93.1±0.006	901.4±0.001	105.5±0.72	8.0±0.04	24.7±0.62	20.2±0.16	1.9±0.43	0.5±0.15	0.7±0.44
DO	56.7±0.002	597.10±0.003	27.4±0.86	3.3±0.23	34.9±0.87	22.3±0.72	2.2±0.54	0.8±0.25	0.8±0.12
ID	76.9±0.008	721.7±0.02	51.6±1.08	2.3±0.10	36.9±1.03	15.0±0.69	0.1±0.35	0.6±0.23	0.7±0.14
HP	66.8±0.005	805.7±0.04	44.8±0.51	6.7±0.64	24.4±0.21	15.6±0.58	1.4±0.17	0.5±0.14	0.6±0.15
PO	48.6±0.003	936.23±0.27	13.9±0.24	2.3±0.14	26.9±0.81	17.0±0.0.31	1.9±0.56	0.6±0.21	0.7±0.12

Values represent mean±SD, n>3. All the experiments were repeated at least three times.

Micronutrients deficiency among individuals with diabetes is a well recognized phenomenon[23]. Role of various micronutrients in metabolic disturbances leading to lifestyle related disorders is amply reported in literatures[24,25]. However, the knowledge about how micronutrients help glycemic control is least explored[26]. Multicomponent-micronutrients analysis of different rice samples is presented in table 2. It was interesting to note that among four analyzed vitamin Bs [Vitamin B_2 (riboflavin), vitamin B_3 (niacin), vitamin B_5 (pantothenic acid), vitamin B_7 (biotin) and vitamin B_9 (folic acid), Table 2], higher concentration of niacin (Vit. B_3, nicotinic acid) was found positively correlated with enzyme inhibitory activity (Fig. 6). Extract from BR and BO rice presented highest concentration of niacin among analyzed rice samples (Table 2). These rice extracts also presented potent enzyme inhibitory activity than other rice's extract (Fig. 1a & 1b). Nicotinic acid (niacin) has been observed to improve disturbed glucose level and parameters of oxidative stress in diabetic animal model[27] and several hybrids of niacin are reported to possess α-glucosidase inhibitory and antihyperglycemic activity[28]. These observations disclose that presence of micronutrients and phytochemicals substantially affect digestion of rice and impart multiple beneficial effects in diabetic conditions. Therefore, selection of micronutrients and phytochemicals rich rice varieties may help control postprandial blood glucose excursion in hyperglycemic population.

Consumption of oxidized (e.g. fried) or oxidizable (e.g. cooked) meal increases burden of oxidative stress[29]. This stat is further aggravated when food is poorly equipped with phytonutriens[30]. Disturbance in redox stat arising due to excess oxidative load or the poor supply of nutritive antioxidants indicates dietary

oxidative stress[30]. Sustained hyperglycemia is a known inducer of postprandial oxidative stress and is associated with the higher risk for atherosclerosis, diabetes, and obesity[31]. In fact, free radicals generated beyond body's normal physiological control lead oxidative stress[32] that overwhelm body's ability to regulate and maintain level balance between physiological oxidants and antioxidants[33]. Therefore, presence of free radicals scavenging antioxidative principles in diet becomes indispensable to pacify postprandial oxidative stress and mitigate free radicals induced damage to biomolecules.

The potentials of sampled rice extract in scavenging of free radicals were estimated applying ABTS$^{\cdot+}$ and DPPH radicals. The ABTS$^{\cdot+}$ cation is amphiphilic in nature and is applied to identify both the hydrophilic as well as lipophilic antioxidants present in dietary materials[34] where as organic nitrogen centered DPPH radical evaluates the reducing power of an antioxidant[35]. Figure 7 presents kinetics of ABTS$^{\cdot+}$ and DPPH radical scavenging activity by extracts is shown in Figure 8. It is evident
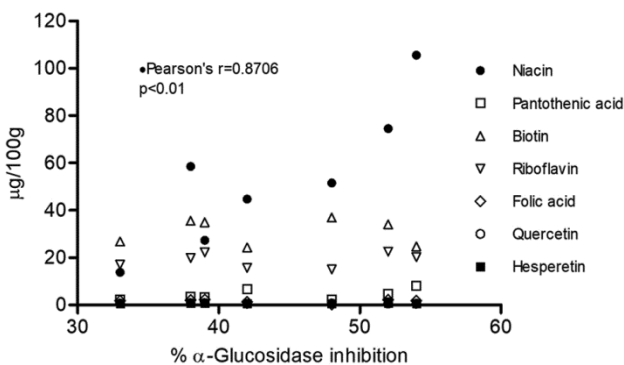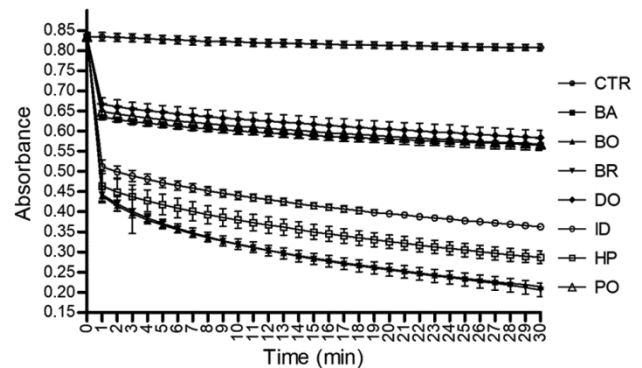
Fig. 7 — Decrease in color absorbance of ABTS$^{\cdot+}$ radical over time due to extract of rice. Steeper the slope faster is the radical scavenging.

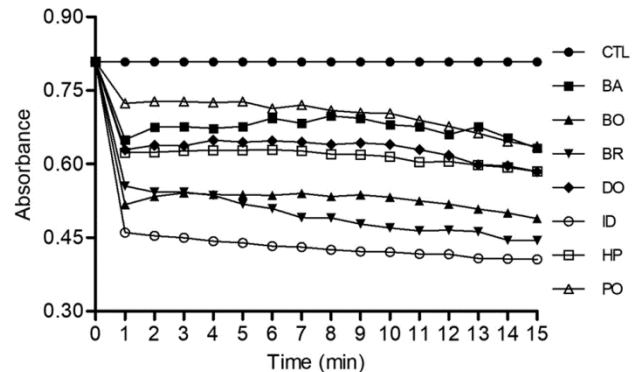Fig. 8 — Decolorization of DPPH radical over time by extract of rice sample. Steeper the slope faster is the radical scavenging.

Fig. 6 — Influence of different micronutrients on intestinal α-glucosidase activity.

from figure 7 that the $ABTS^{\cdot+}$ radical scavenging potential of extract from different rice samples varied largely. In the order of $ABTS^{\cdot+}$ cation scavenging activity, extract of BR and BO rice with 74% scavenging at 30th minute stood most potent followed by HP (65%), ID (55%), BA and PO (30%), and DO extract was least potent with 28% radical scavenging (Fig.7). On the other hand, the order of DPPH radical scavenging activity of extracts (Fig. 8) was not the same as found for $ABTS^{\cdot+}$ radical scavenging. In this case ID rice extract with 50% DPPH scavenging activity at 15th minute stood first followed by BR (46%), BO (41%), HP and DO (29%). BA and PO rice extract were found least potent in scavenging DPPH radical with 24% activity (Fig. 8). Although, these radicals ($ABTS^{\cdot+}$ and DPPH) scavenging activity was positively correlated with polyphenol content in rice extract (Fig. 9) and vitamin B_3 (niacin, Fig. 10) the differences in activity potentials may be ascribed to the differences in micronutrients phytochemicals types, compositions and concentrations in different rice varieties.
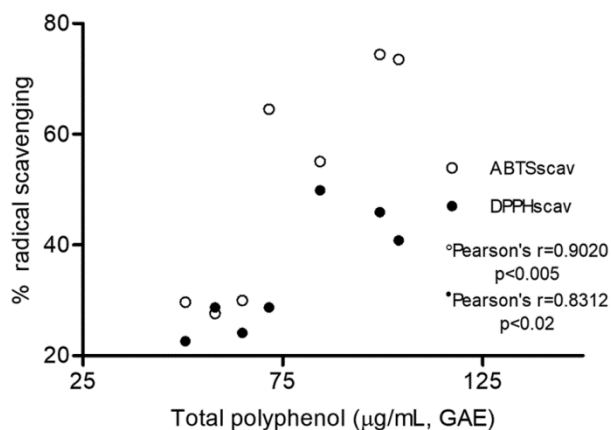

Fig. 9 — Correlation between polyphenol content in rice extract with free radicals scavenging activity.
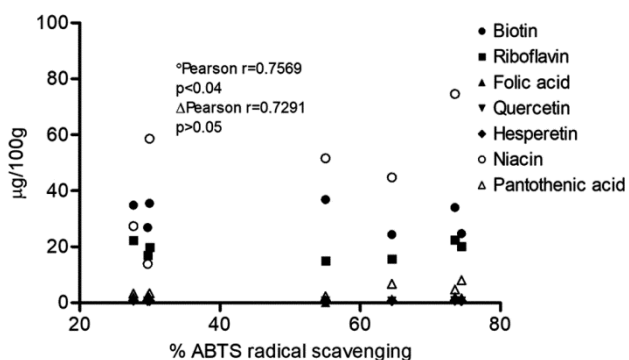

Fig. 10 — Correlation between micronutrient vitamin content in rice and $ABTS^{\cdot+}$ radical scavenging activity.

Table 3 presents ranking of rice depending upon their ability to slow down carbohydrate digestion, $ABTS^{\cdot+}$ and DPPH radical scavenging potentials. It is evident from table 3 that based on the required qualities to slow down carbohydrate digestion with substantial free radicals scavenging potentials, only BR and BO could meet maximum criteria. However, each variety of rice has its own important quality that can be utilized depending upon the necessity.

In a recently reported randomized controlled clinical trial[36], it was found that replacement of parboiled brown rice at the place of white rice for three months, in the diet of study participants possessing symptoms of metabolic syndrome significantly improved HbA1c level. Improvement in HbA1c, total cholesterol and low density lipoprotein-cholesterol was noticed in participants with BMI ≥25. Improvements in parameters of inflammation were also recorded in study participants with brown rice in the study[36]. Observations made in our study provide in part, presence of phytochemicals micronutrients and plausible mechanism of actions by which parboiled brown rice imparted beneficial effects to the study participants.

In conclusion, our study reveals that although more carbohydrate and starch content in rice is responsible for higher postprandial glycemic excursion, their digestion is controlled by number of micronutrients and phytochemicals. Selection of micronutrient and polyphenol rich rice therefore, may help counter rapid hyperglycemic excursion induced due to rice consumptions. Our study also supports clinical observations that consumption of brown/parboiled rice may offer therapeutic benefits to people with metabolic syndrome and high BMI values. Further studies are required to explore the role of particular micronutrients and phytochemicals responsible for management of postprandial glycemic excursion present in different varieties of rice.

Table 3 — Ranking of rice sample based on activities		
Rank	Decreasing α-glucosidase activity	$ABTS^{\cdot+}$ scavenging activity	DPPH scavenging activity
1	BR (54%)	BR (74%) & BO (74%)	ID (50%)
2	BO (52%)	HP (65%)	BR (46%)
3	ID (48%)	ID (55%)	BO (41%)
4	HP (42%)	BA (30%) & PO (30%)	HP (29%) & do (29%)
5	DO (40%) & BA (39%)	DO (28%)	BA (24%) & PO (24%)
6	PO (31%)		

Acknowledgement

Conflict of Interest

All the authors declare that they have no conflict of interest financial or otherwise.

Author Contribution

SD, KA, AA & AM contributed in experimental analysis, methodology, data generation, interpretation and preparation of manuscript drafts. KSB, MKRM & AKT were involved in concept development, experiment design, data analysis, data interpretation, manuscript writing, editing, finalization and funding arrangements.

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