

## Identification of proglycemic and antihyperglycemic activity in antioxidant rich fraction of some common food grains

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**Abstract:** Methanolic extract of some common food grains were evaluated for antioxidant activities with respect to their total polyphenolic and anthocyanins content, prevention of ABTS oxidation, scavenging of free radicals ABTS<sup>+</sup>, DPPH, and H<sub>2</sub>O<sub>2</sub>. *In vitro* inhibition of rat intestinal  $\alpha$ -glucosidase and formation of AGEs were also studied. These extracts were further evaluated for their influence on blood glucose level in normal rats with starch tolerance test. Polyphenolic content was observed in all the extracts. Bran rice, and black gram yielded maximum amount. Anthocyanins were detected in bran rice, Bengal gram, black gram and green pea extracts. All the extracts displayed varying degrees of ABTS<sup>+</sup> radical scavenging activity. A significant positive correlation between polyphenolic content and ABTS<sup>+</sup> radical scavenging activity was observed. Black gram, bran rice, pearl millet, and white gram extracts mitigated ABTS oxidation also. Bengal gram, pearl millet, black gram, white gram and green pea scavenged H<sub>2</sub>O<sub>2</sub>. Bran rice and polished rice extracts were potent in scavenging DPPH radical. Pearl millet, black gram and bran rice inhibited formation of AGEs. Except polished rice, other extracts displayed very mild  $\alpha$ -glucosidase inhibition. Interestingly, pearl millet, white gram, black gram and white rice extracts despite the presence of antioxidant properties displayed pro-glycemic pattern. However, in addition to antioxidant activities, bran rice, barley and Bengal gram extracts exhibited antihyperglycemic property. This is the first report identifying proglycemic and antihyperglycemic activity in antioxidant rich fraction from food grains.

**Keywords:** Food grains, free radical scavenging, antioxidants,  $\alpha$ -glucosidase inhibition, proglycemic, antihyperglycemic activity

### Introduction

Diabetes mellitus is one of the most common chronic diseases in nearly all countries, and continues to increase in number and significance, as changing lifestyle lead to reduced physical activity, and increase in obesity (Shaw *et al.*, 2010). The hallmark of type-2 diabetes mellitus is insulin resistance as well as pancreatic  $\beta$ -cell dysfunction (Kaneto *et al.*, 2010) and hyperglycemia has been the main diagnostic and therapeutic target for diabetes mellitus (Tiwari *et al.*, 2008; Raju *et al.*, 2010). Changes in dietary pattern have been held responsible for high plasma glucose levels and increased prevalence of insulin resistance in diabetics (Wolver and Mehling, 2003). Highly processed, calorie-dense, nutrient depleted diet leads to exaggerated postprandial spikes in blood glucose and lipids that induces immediate oxidative stress. Induction of oxidative stress has been observed to increase in direct proportion to the increase in postprandial blood glucose level (O'Keefe *et al.*, 2008). Postprandial hyperglycemia (PPHG) has emerged as a prominent and early defect in ensuing type-2 diabetes mellitus (Carroll *et al.*,

2003) and an independent predictor of development of future cardiovascular events even in non-diabetic individuals (O'Keefe *et al.*, 2008). Furthermore, PPHG and PPHG induced oxidative stress has also been accounted for the pathogenesis of all diabetic complications (Brownlee, 2001). In fact, under hyperglycemic condition, non-enzymatic glycation of amino group of proteins with aldehydic group of reducing sugar leads to the formation of number of compounds commonly referred to as advanced glycation end products (AGEs). These AGEs are held responsible for development of diabetic complications and advancing the aging process (Guglicci, 2000).

Evidences are increasingly evolving to show that traditional diets like Okinawa (Lienchtenstein *et al.*, 2006), Mediterranean (Fito *et al.*, 2007), or the Indian (Tiwari, 2008) that were minimally processed, low in caloric density, high in nutrient density and rich in antioxidants may offer preventive as well as therapeutic benefits in combating postprandial hyperglycemic excursion (PPHGE) and oxidative stress induced damage (Lienchtenstein *et al.*, 2006). Paradoxically however, it has been advised that antioxidants if taken on an empty stomach, may

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increase appetite, and when taken with food, may affect satiety (Diano, 2009). Furthermore, it has recently been observed that consumption of some antioxidant rich fruits and fruits juice significantly and adversely affect lipids level of individuals (Alvarez-Parrilla *et al.*, 2010). However, there is no information about the effect of antioxidant rich dietary food grains on blood glucose level.

In the light of above observations it would be prudent therefore, to explore dietary commodities that possess potent antioxidant activities and simultaneously mitigate PPHGE, and segregate those that may increase blood glucose level. Identification of such food grains may offer dual benefit in combating meal induced PPHGE as well as PPHG induced oxidative stress. Therefore, the aim of our study was to analyze antioxidative properties of some common food grains and evaluate their effect on blood glucose level in normal rats with starch tolerance test.

## Material and Methods

### Food grains

Whole grains of pearl millet (*Pennisetum glaucum* Linn.), barley (*Hordeum vulgare* Linn.), whole rice (rice with bran) and polished rice i.e. dehusked polished white rice (*Oryza sativa* Linn.), Bengal gram and white gram [also called Kabuli chana] (*Cicer arietinum* Linn.), black gram (*Phaseolus mungo* Linn.) and green pea (*Pisum sativum* Linn.) were purchased from the local markets of Hyderabad city (India).

### Extraction of antioxidant rich fraction

Extraction of antioxidant rich fraction was carried out as described by Yao *et al.* (2010) with 85% methanol acidified with 1.0N HCl for 24 h at room temperature. The supernatant was vacuum filtered, concentrated to 1/3 volume under reduced pressure in rotary evaporator (50±1°C) and lyophilized to dry. Extracts were stored refrigerated until analysis.

### Determination of total phenolic content

Total phenolic content in the extracts was measured using Folin-Ciocalteu reagent (Yao *et al.*, 2010). Briefly, 25 µL (5 mg/ml DMSO solution) of the extract was diluted with 2.5 mL of distilled deionised water followed by addition of 250 µL of Folin-Ciocalteu reagent (1M) and 250 µL of Na<sub>2</sub>CO<sub>3</sub> (20%, w/v). After incubation for 60 min at room temperature absorbance was measured spectrophotometrically at 765 nm (BioTek<sup>synergy4</sup> multi-mode microplate reader, BioTek Instruments, Inc). Quantification was

performed with respect to the standard curve of gallic acid. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the extract. All determinations were performed in triplicates.

### Determination of total anthocyanins

Presence of anthocyanins in the extracts was determined as described by Giusti *et al.* (1999). Samples were dissolved in 25 mM potassium chloride solution (p<sup>H</sup>1.0) and 0.4 M sodium acetate buffer (p<sup>H</sup> 4.5). Absorbance was measured at 510 and 700 nm (Perkin Elmer<sup>precisely</sup> Lambda 25,UV/VIS spectrometer). Data was expressed as milligrams of anthocyanins per 100 g of extract using a molar extinction coefficient of 26900, molecular weight of 449.2, and an absorbance of  $A=[(A_{510}-A_{700}) p^{H1.0}-(A_{510}-A_{700}) p^{H4.5}]$ . All determinations were performed in triplicates.

### ABTS<sup>+</sup> scavenging assay

Scavenging of ABTS<sup>+</sup> cation radical was performed as described by Walker and Everette (2009) with suitable modifications. Briefly, 100 mL stock solution of ABTS<sup>+</sup>(0.5 mM) was prepared by addition of 1 mL potassium persulfate [6.89 mM in PBS (p<sup>H</sup> 8.0)]. Mixture was stored in dark for 16hrs. 10 µL of test sample (5 mg/mL in PBS) was added to 190 µL of ABTS<sup>+</sup> in 96-well microplate. Kinetics of ABTS<sup>+</sup> scavenging was recorded at 734 nm every minute for 1 h using a kinetics programme on BioTek<sup>synergy4</sup> multi-mode microplate reader. For each test sample a separate blank sample (devoid of ABTS<sup>+</sup>) was used for background subtraction. Trolox was taken as standard. Experiments were repeated thrice. For the estimation of ABTS<sup>+</sup> scavenging concentration 50% (SC<sub>50</sub>) various dilutions of the test samples were prepared and end-point absorbance (734 nm) was measured after 1h incubation in the dark. Percentage scavenging of ABTS<sup>+</sup> by test samples was calculated as follows  $[(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test}}) / \text{Absorbance}_{\text{control}}] \times 100$ . Suitable regression analysis was applied for calculation of SC<sub>50</sub>.

### ABTS oxidation assay

For determination of ABTS oxidation a mixture of ABTS liquid substrate and AzBTS microwell enhancer solution in the ratio of 10:1 was prepared according to manufacturer's instruction and stored in the dark. To 160 µL of this mixture, 20 µL of test extract (5 mg/mL in PBS) was added in 96-well microplate. Oxidation of ABTS mixture was initiated by addition of 20 µL of potassium persulfate (6.89 mM, p<sup>H</sup> 8.0). Kinetics of ABTS oxidation was recorded at 734 nm for 30 min at the intervals of 35 sec as above.

Trolox was taken as standard. For the determination of concentration that inhibit 50% ABTS oxidation by test extracts ( $IC_{50\%}$ ) various dilutions of extracts were prepared and end-point absorbance was measured after 30 min incubation in the dark. Calculations were done as above.

#### *H<sub>2</sub>O<sub>2</sub> scavenging assay*

Determination of H<sub>2</sub>O<sub>2</sub> scavenging activity was performed as described by Wettasinghe and Shahidi (1999). To 600  $\mu$ L of H<sub>2</sub>O<sub>2</sub> solution (43 mM H<sub>2</sub>O<sub>2</sub> in 0.1M phosphate buffer (p<sup>H</sup>7.4)), 20  $\mu$ L of test sample (5mg/mL prepared in same buffer) was added. Absorbance values (230 nm) of the reaction mixture was recorded at 0 min and then at every 10th min up to 40 min on Perkin Elmer <sup>precisely</sup> Lambda 25,UV/VIS spectrometer. For each test sample a separate blank sample (devoid of H<sub>2</sub>O<sub>2</sub>) was also used for background subtraction. Percentage of H<sub>2</sub>O<sub>2</sub> scavenging was calculated applying following formula  $[(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test}}) / \text{Absorbance}_{\text{control}}] \times 100$ .

#### *Scavenging of DPPH free radical*

Assay for the scavenging of free radical DPPH was done as reported earlier (Rao *et al.*, 2009). Briefly, in a 96-well microplate, 20  $\mu$ L of test sample (5 mg/mL in DMSO), 100  $\mu$ L of 0.1M tris-HCl buffer (p<sup>H</sup> 7.4) and 100  $\mu$ L DPPH solution (0.5 mM in methanol) were added. The reaction mixture was shaken well. Kinetics of DPPH decolorisation was recorded (517 nm) on BioTek<sup>synergy4</sup> multi-mode microplate reader for 30 min at the intervals of 35 sec. Percentage DPPH scavenging by test samples was calculated applying formula mentioned above.

#### *$\alpha$ -Glucosidase inhibition assay*

Inhibition of rat intestinal  $\alpha$ -glucosidase enzyme was done as reported earlier (Rao *et al.*, 2009). Briefly, in a 96-well microplate 20  $\mu$ L of test sample (10 mg/ml DMSO) was incubated with 50  $\mu$ L of crude intestinal  $\alpha$ -glucosidase for 5 min and then reacted with 50  $\mu$ L of substrate (5 mM, p-nitrophenyl-  $\alpha$ -D-glucopyranoside in 100 mM phosphate buffer p<sup>H</sup>, 6.8). Kinetics of release of p-nitrophenol was measured spectrophotometrically (BioTek<sup>synergy4</sup> multi-mode microplate reader) for 15 min at the intervals of 35 sec at 405 nm. Percent enzyme inhibition was obtained applying formula mentioned above.

#### *In vitro glycation of protein*

Glycation of BSA protein was performed as described by Sultana *et al.* (2009) in sterilized Eppendorf tubes with suitable modifications. Briefly,

each Eppendorf tube contained 620  $\mu$ L reaction mixtures (200  $\mu$ L BSA (10 mg/mL), 200  $\mu$ L glucose anhydrous (50 mg/ml), 200  $\mu$ L sodium phosphate buffer (67 mM, p<sup>H</sup> 7.4) and 20  $\mu$ L of test sample dissolved in DMSO. Glycated control contain 200  $\mu$ L BSA, 200  $\mu$ L glucose, 200  $\mu$ L sodium phosphate buffer and 20  $\mu$ L DMSO, while blank contains 200  $\mu$ L BSA, 400  $\mu$ L sodium phosphate buffer, 20  $\mu$ L DMSO. Reaction mixture was incubated at 37°C for 7 days. After incubation of BSA protein-glucose mixture for 7 days, 60  $\mu$ L of tri-chloro acetic acid (100%) was added in each eppendorf tube and centrifuged (15,000 rpm) for 4 min at 4°C. After centrifugation the pellets were washed with 600  $\mu$ L of tri-chloro acetic acid (10%). The supernatant containing glucose, inhibitor and interfering substance was removed. Pellets containing AGE-BSA were dissolved in 600  $\mu$ L PBS.

#### *Analysis of Fructosamines*

Extent of glycation of protein under the influence of test samples was analyzed by measuring formation of fructosamines as described by Wu *et al.* (2009). Briefly, 200  $\mu$ L glycated material and 800  $\mu$ L of NBT reagent [300  $\mu$ M in sodium carbonate buffer (100 mM, p<sup>H</sup> 10.35)] was incubated at ambient temperature for 15 min, and absorbance was measured spectrophotometrically (BioTek<sup>synergy4</sup> multimode microplate reader, BioTek Instruments, Inc. USA) at 530 nm against a blank. All the determinations were carried out in triplicates. Percent inhibition and  $IC_{50}$  values (50% inhibitory concentration of test compounds) were obtained accordingly.

#### *Measurement of fluorescent AGEs formation*

The fluorescence intensity of glycated protein was measured fluorophotometrically (BioTek<sup>synergy4</sup> multi-mode microplate reader, BioTek Instruments, Inc. USA) at an excitation wavelength of 370 nm and an emission wavelength of 440 nm as described by Wu *et al.* (2009) as a measure of AGEs. All the determinations were carried out in triplicates. Percent inhibition and  $IC_{50}$  values (50% inhibitory concentration of test compounds) were obtained as described above.

#### *Starch tolerance test*

Antihyperglycemic activity in methanolic extract of the food grains was determined following starch tolerance test (Tiwari *et al.*, 2008). Male Wistar rats (190-200 g body weight) were obtained from National Institute of Nutrition (CPCSEA Reg.No.154, Government of India), Hyderabad. Rats were housed in standard polyvinyl cages in institute's animal house. Room temperature was maintained at 22 $\pm$ 1°C

with an alternate 12 h light dark cycle. Food and water were provided ad libitum. The Institutional Animal Ethical Committee approved experimental protocols. Rats were divided into various groups of 5 rats in each group. All the animals were kept for overnight fasting. Next day forenoon blood was collected from retro orbital plexus in EDTA containing tubes. Basal plasma glucose level ('0' h) was estimated by glucose-oxidase method by auto blood analyzer (Bayer EXPRESS PLUS). Test samples were suspended in normal saline and administered orally (1000 mg/kg body weight). Control groups of animals were given normal saline. Acarbose was taken as standard drug (10 mg/kg body weight). Fifteen minutes after oral administration of test samples and standard drug, animals were given soluble starch (2 g/kg body weight). Blood was collected at the intervals of 30, 60, 90 and 120<sup>th</sup> min post starch feeding. Plasma glucose levels were measured as described above. Area under the curve (AUC) as a measure of postprandial hyperglycemic load was calculated following Trapezoidal rules (Raju *et al.*, 2010).

#### Statistical analysis

The Pearson correlation coefficient (R) and p-value were used to show correlation and significance between total polyphenols and free radical scavenging activity. One way ANOVA followed by Dunnett's Multiple comparison test was applied to compare difference in animal study groups.

## Results

#### *Yield of methanolic extract, total polyphenolics and anthocyanins*

The yield of methanolic extracts obtained from eight grains is presented in Table 1. Black gram produced maximum yield followed by Bengal gram. Green pea and polished rice produced lowest yield. Pearl millet and white gram, barley and bran rice produced equal amounts of methanolic extract.

Maximum polyphenol content was obtained in bran rice followed by black gram. Barley contained lowest amount of polyphenol. Anthocyanins were detected in bran rice, Bengal gram, black gram and green pea extracts. There was no correlation between the yield of methanolic extract and polyphenol content (Pearson  $r = 0.3724$ ,  $p > 0.05$ , two tailed).

#### *ABTS<sup>+</sup> scavenging activity*

Figure 1(a) presents ABTS<sup>+</sup> scavenging potentials of extracts. Black gram was observed most potent in scavenging ABTS<sup>+</sup> radical. Bengal gram and white gram displayed similar radical scavenging potential.

**Table 1.** Percentage yields, TPC, and TAC of methanolic extracts

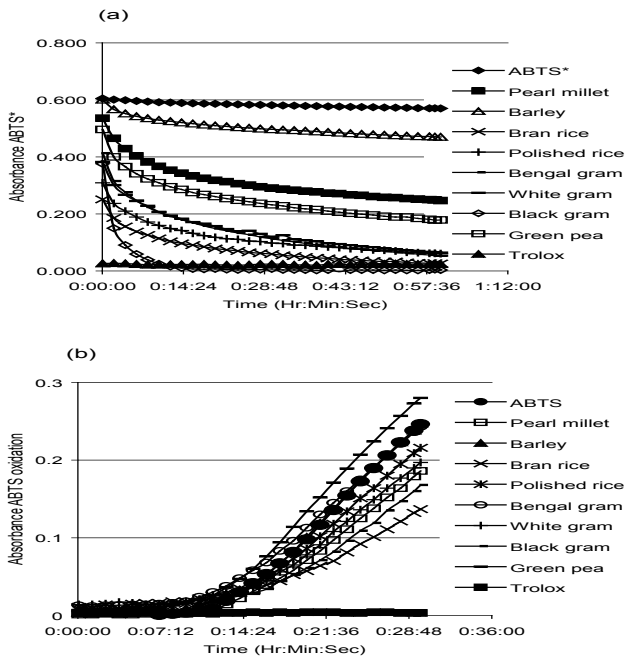
| Test samples  | %Yield (w/w) | TPC | TAC  |
|---------------|--------------|-----|------|
| Pearl millet  | 4.5          | 23  | ND   |
| Barley        | 2.9          | 7   | ND   |
| Bran rice     | 2.6          | 90  | 0.19 |
| Polished rice | 1.2          | 33  | ND   |
| Bengal gram   | 8.4          | 34  | 0.12 |
| White gram    | 4.5          | 44  | ND   |
| Black gram    | 9.5          | 82  | 0.05 |
| Green pea     | 1.9          | 12  | 0.01 |

Percentage of yield represents gm/100gm of the raw materials. TPC [total polyphenolic content] was expressed as gallic acid equivalent mg/100g of the extract. TAC (total anthocyanins content) was measured as milligram anthocyanins/100gram extract. Values represent mean of triplicate determinations in case of % yield, TPC, TAC. ND; not detectable.

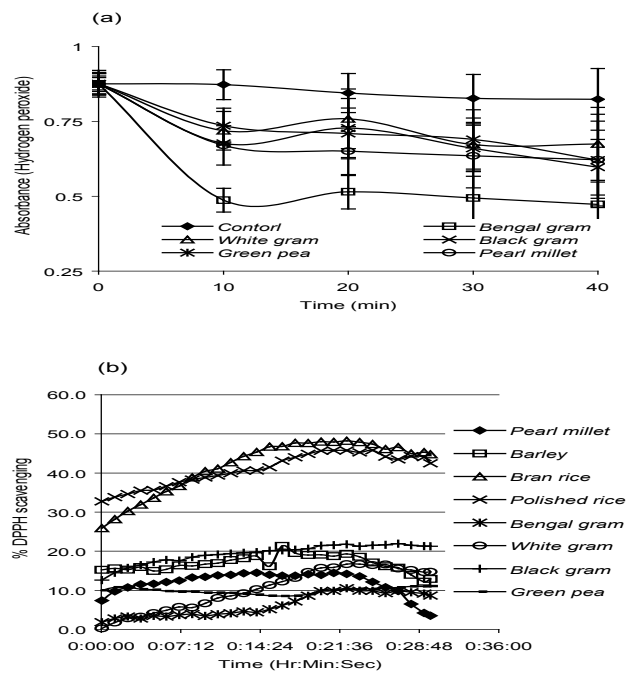
Green pea extract however, was observed moderate ABTS<sup>+</sup> radical scavenger. Bran rice was double potent than polished rice in scavenging ABTS<sup>+</sup> radical (Figure 1a). In the order of their 50% ABTS<sup>+</sup> radical scavenging capacity (SC<sub>50%</sub>), black gram (6 µg/mL) was more potent than bran rice (12.4 µg/mL) followed by Bengal gram (22.4 µg/mL), white gram (23.1 µg/mL), polished rice (26.6 µg/mL), green pea (35.0 µg/mL), pearl millet (52.4 µg/mL) and barley (102.9 µg/mL). It was observed that total phenolic content was directly associated with SC<sub>50%</sub> values for ABTS<sup>+</sup> scavenging (Pearson  $r = 0.7293$ ,  $p < 0.04$ , two tailed). None of the extracts displayed scavenging potency close to standard compound Trolox. Biphasic kinetic patterns were observed in our study for some extracts (Figure 1a). Initially, black gram displayed fast scavenging rate that became stable after fifteen minutes, however, bran rice, polished rice, Bengal gram, white gram, green pea and pearl millet could not reach stable end point even after one hour. Barley extract displayed slow kinetics. Trolox exhibited the fastest kinetics, which reached end point within seconds.

#### *Mitigation of ABTS oxidation*

Figure 1(b) displays kinetics of ABTS oxidation induced by potassium persulphate. Barley and Bengal gram extracts could not protect ABTS oxidation. Green pea extract was observed to further oxidation of ABTS in addition to potassium persulphate. Bran rice displayed strong antioxidative activity followed by black gram, pearl millet and white gram against potassium persulphate induced ABTS oxidation. The antioxidative potential of bran rice was observed more potent than polished rice (Figure 1b). Black gram (IC<sub>50%</sub>; 70.3 µg/mL) displayed strong antioxidant activity, which was twice that of the white gram extract (IC<sub>50%</sub>; 168.4 µg/mL). Similar results were obtained for bran rice extract (IC<sub>50%</sub>; 77.7 µg/mL) and pearl millet (IC<sub>50%</sub>; 139.8 µg/mL).



**Figure 1.** Kinetics of ABTS + Scavenging (a) and oxidation of ABTS by potassium persulphate (b) under the influence of different extracts and standard antioxidant compound Trolox. Time course of the absorbance changes for reaction of the extracts and Trolox was used. Both figures represent observation made with 50- $\mu\text{g}/\text{mL}$  concentration of the respective extract. Values represent mean of the triplicate observations. Time scale represents (Hour: Minutes: Seconds).



**Figure 2.** Effect of methanolic extracts in scavenging hydrogen peroxide (a) and DPPH radical (b) with respect to time. 100- $\mu\text{g}/\text{mL}$  concentration of each extract was used in the assay. Values represent mean $\pm$ SD of triplicate observations in case of hydrogen peroxide and means (triplicate observations) in the study of % DPPH scavenging. Time scale for figure (b) represents (Hour:Minutes:Seconds).

### Scavenging of $\text{H}_2\text{O}_2$

$\text{H}_2\text{O}_2$  scavenging activity of the extracts did not differ significantly from the activity at 10<sup>th</sup> min to over 40<sup>th</sup> min (Figure 2a). Bran rice, polished rice and barley extracts could not display  $\text{H}_2\text{O}_2$  scavenging activity. Though none of the extracts could scavenge  $\text{H}_2\text{O}_2$  more than 50% at 100  $\mu\text{g}$  primary screening concentration, extract of Bengal gram was found more potent (43% scavenging of  $\text{H}_2\text{O}_2$ ) than black gram (28%), green pea (25%), pearl millet (25%), and white gram (18%).

### DPPH free radical scavenging

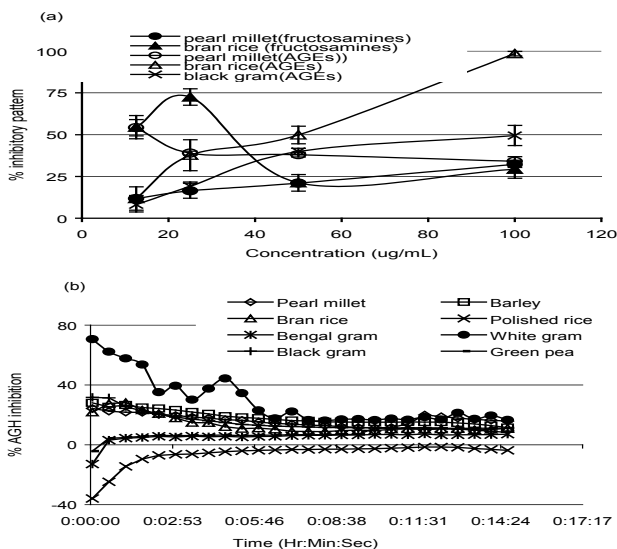
Both, bran rice and polished rice extracts displayed comparable DPPH scavenging pattern (Figure 2b). 45% DPPH radical scavenging activity for bran rice and 42% for polished rice extract was observed at the end of 30 min. Other extracts displayed mild DPPH scavenging activity. DPPH scavenging activity of pearl millet and barley extract decreased at the end of 30 min. Green pea extracts displayed constant DPPH scavenging activity. White gram showed linear increase in scavenging activity. Similar pattern was observed for Bengal gram; however, it was less potent than white gram. Black gram scavenged 21% DPPH at the end of 30 min at 100  $\mu\text{g}/\text{mL}$  concentration (Figure 2b).

### AGEs formation and $\alpha$ -Glucosidase inhibition

Figure 3(a) presents AGEs formation inhibitory activity of extracts. Pearl millet extract showed mild fructosamine inhibitory activity with  $\text{IC}_{50}$  value of 178.5  $\mu\text{g}/\text{mL}$ . Bran rice extract displayed potent fructosamine inhibitory activity at lower concentration however; it decreased with increasing extract concentration. Similarly, bran rice and black gram extracts showed concentration dependent inhibition in fluorescent AGEs formation ( $\text{IC}_{50}$  value for bran rice 47.5  $\mu\text{g}/\text{mL}$  and 92.3  $\mu\text{g}/\text{mL}$  for black gram extract). Pearl millet extract displayed pattern like bran rice as in the case of fructosamine formation (Figure 3a).

Figure 3b represents kinetic pattern of rat intestinal  $\alpha$ -glucosidase inhibition by extracts. Values above '0' on the Y-axis represent inhibition of the enzyme activity while below, acceleration in the activity. It was observed that polished rice initially accelerated  $\alpha$ -glucosidase activity, which was stabilised over 8 min and paralleled close to control values (X-axis). Initially, enzyme activity was observed slow under the influence of white gram. At the end of 15 min of incubation of substrate with enzyme and extract mixture, pearl millet and white gram extracts displayed 16% to 17% enzyme inhibition respectively followed by barley, bran rice and green pea extracts displaying identical enzyme inhibitory activities

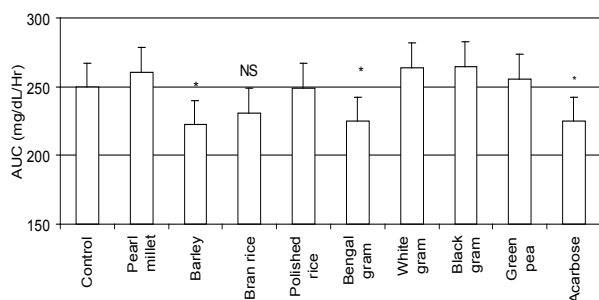
(Figure 3b). Bengal gram showed lowest inhibitory activity (7%) at the end of 15 min. Results show that extracts possess very mild enzyme inhibitory potential.



**Figure 3.** Advanced glycation end products inhibitory pattern of methanolic extract of food grains (a) and (b) Kinetics of rat intestinal  $\alpha$ -glucosidase (AGH) inhibition by methanolic extracts of food grains (concentration 100  $\mu$ g/mL). Time course of the changes in percentage of inhibition of the enzyme by extracts was used. Values above scale '0' on Y-axis represent percent inhibition of the enzyme  $\alpha$ -glucosidase by extracts over time and vice versa below the scale '0' on Y-axis. Values represent mean inhibition of the triplicate. Time scale represents (Hour: Minutes: Seconds).

**Antihyperglycemic activity**

Starch feeding induced sharp rise in plasma glucose level of rats (Figure 4). In terms of starch induced glycemic load, the area under the curve (AUC, mg/dL/hr) shows that barley and Bengal gram displayed significant antihyperglycemic activity ( $p < 0.05$ ) however; the degree of significance could not be obtained for bran rice extract. A pro-glycemic behaviour was observed with other extracts.



**Figure 4.** Antihyperglycemic activity of methanol extract of food grains under starch-induced hyperglycemia in normal rats. Area under the curve (AUC, mg/dL/hr) represents per hour postprandial glycemic load. ANOVA followed by Dunnett's multiple comparison tests was applied to compare difference between the groups. \* $P < 0.05$  when compared with control. NS (not significant). 1000-mg/kg body weight dose of extracts and 10-mg/kg-body weight dose of standard  $\alpha$ -glucosidase inhibitor antihyperglycemic drug, Acarbose was used in the study. Data represent mean  $\pm$  SD, N=5.

**Discussion**

High carbohydrate meal induces higher postprandial glucose levels, decreases total radical-trapping antioxidant parameters and renders oxidative damage to biomolecules (Ceriello *et al.*, 1999). Therefore, antioxidant therapy may be of great interest in diabetic patients in order to prevent and/or reduce the development of oxidative stress induced by PPHGE.

Although there is no consensus regarding selection of solvent that extract absolute antioxidants from natural resources like medicinal plant materials, it has been observed that in comparison to other solvents, methanol extract yields relatively higher antioxidant activities (Sosulski *et al.*, 1982; Oki *et al.*, 2002). Therefore, we selected methanol as extraction solvent in our study. The yield of methanolic extracts obtained from eight grains range from 1.2% for the polished rice to 9.5% for black gram. Phenolic compounds (Huang *et al.*, 2009) and anthocyanins (Wang *et al.*, 1997) are considered as the major compounds that contribute to the total antioxidant activities present in food grains. Anthocyanins were detected in bran rice but not in polished white rice. These observations approve the notion that milling process removes significant amount of polyphenolic antioxidants from the raw grains (Tiang *et al.*, 2004). Our study observed high yield of polyphenols in black gram and bran rice. Anthocyanins are colored compounds. White gram, barley and pearl millet are not colored grains. This may be the reason anthocyanins could not be detected in these materials.

It has been observed that antioxidant activities of substances tested on different model systems have produced diverse results (Huang *et al.*, 1996). A particular antioxidant may promote formation of hydroperoxides at early stages of the oxidation, and may inhibit formation of secondary oxidation products at the later stages (Frankel *et al.*, 1996). Furthermore, antioxidant activity of a test substance may differ when it is tested on pre-formed radicals originating in different environment, and also when studied in a model system where free radical is generated simultaneously in the test system. Therefore use of more than one method is recommended to give a comprehensive analysis of antioxidative efficiency of test substance under the study (Dini *et al.*, 2009).

Niki *et al.* (1995) suggested that considering the activities of radicals and substrates present in biological system, peroxy radicals should be the major target for radical scavenging antioxidants. The ABTS test model is based on formation of ABTS<sup>+</sup> cation either oxidized by potassium persulphate (Re

*et al.*, 1999), lactoperoxidase, myeloperoxidase, metmyoglobin or  $H_2O_2$  (Reszka and Britigan, 2007).  $ABTS^+$  represents free radical model originating in hydrophilic medium (Re *et al.*, 1999) and serves as an excellent substrates for peroxidases (Re *et al.*, 1999; Reszka and Britigan, 2007). DPPH is a nitrogen-centered free radical that reacts similar to peroxy radical (Wright, 2003; Tiwari, 2004) and has been treated as a free radical model originating in lipophilic medium (Hatano *et al.*, 1989).

Our study observed that methanol extract of black gram followed by bran rice was most potent in scavenging  $ABTS^+$  radical and mitigating oxidation of ABTS induced by potassium persulphate. Extract of white gram and pearl millet also mitigated ABTS oxidation. Though Bengal gram, polished rice, green pea and barley displayed potent  $ABTS^+$  scavenging activity, they could not mitigate oxidation of ABTS in our study. Bran rice and polished rice extracts showed moderate DPPH scavenging activity while other extracts displayed mild DPPH scavenging potentials, pearl millet being the least potent. High DPPH scavenging activity of bran rice and polished rice extracts may be due to the presence of lipid soluble antioxidants such as vitamin E and  $\gamma$ -oryzanol (Xu *et al.*, 2001). A strong correlation between polyphenolic content and  $ABTS^+$  scavenging activity was observed (Pearson's correlation  $R=0.7293$ ,  $P<0.04$ ). The nature and content of polyphenols present in the extracts may be held responsible for different degree of antioxidant activities in different methods.

$H_2O_2$  at micromolar concentration is poorly reactive however, it generates hydroxyl radical ( $\cdot OH$ ) in the presence of metal ions and oxygen. Therefore,  $H_2O_2$  scavenging processes are important in living organisms (Aruoma, 1998). Phenolic compounds may accelerate conversion of  $H_2O_2$  to  $H_2O$  (Wettasinghe and Shahidi, 1999). In our study only Bengal gram extract displayed potent  $H_2O_2$  scavenging activity. The concentration of  $H_2O_2$  dropped sharply during the initial 10 minutes period of the assay. Other extracts like white gram, pearl millet, green pea and black gram were less potent.

In the early stage of the non-enzymatic glycation of proteins, reducing sugars react with free amino group of protein to form Schiff base and then produce stable ketoamine also called fructosamine (Wu *et al.*, 2009). These ketoamines reduce NBT in alkaline solution to yield a colored monoformazon dye with absorbance maxima at 530 nm (Johnson *et al.*, 1982). In our study pearl millet extract displayed slow but concentration dependent increase in inhibition of fructosamine formation. Bran rice extract however, showed reverse trend. Similarly, bran rice

and black gram extracts displayed concentration dependent increase in inhibiting AGEs formation, the pearl millet showed reverse trend. These trends in concentration-dependent effects have been observed by earlier workers also and may not be interpreted in a straightforward manner (Baker *et al.*, 1994). Ou and Wolff (1993) have reported that aminoguanidine a known inhibitor of AGEs formation possess prooxidant activity at higher concentration in physiological buffer, which may contribute to increase in the color formation in fructosamine assay (Baker *et al.*, 1993). The non-linear response with these extracts in our study therefore, may be explained due to increase in radical trapping activities at lower concentration and then possible prooxidant activity at higher concentration.

Inhibition of  $\alpha$ -glucosidase, an enzyme present abundantly at the brush border of small intestine, has been found to delay carbohydrate digestion, absorption, and diminish PPHG level (Lam *et al.*, 1998; Rapisis and Dimitriadis, 2001; Dimitriadis *et al.*, 1991). None of the extract in our study however, could display potent  $\alpha$ -glucosidase inhibition.

Slama *et al.* (2006) argue that postprandial glycemic excursion plays an important role in total hyperglycemia and advocates postprandial delta-glycemia as a more useful tool than the conventional examination of absolute postprandial rise in blood glucose level. Barley, Bengal gram and bran rice extracts displayed potent antihyperglycemic activity and their activity was comparable to the standard drug Acarbose. Significant antihyperglycemic activity by barely and Bengal gram extracts in starch tolerance tests in our observations may not be attributed to their mild *in vitro*  $\alpha$ -glucosidase inhibition, however, may find explanation in part, by virtue of the insulin secretagogue activity of polyphenols and anthocyanins (Jayaprakasam *et al.*, 2005). It is important to note that regardless of the presence of antioxidant properties, pearl millet, white gram, black gram and white rice extract displayed pro-glycemic pattern. To the best of our knowledge, this is the first report identifying antihyperglycemic activity in antioxidant rich fraction of barley and Bengal gram, and pro-glycemic attitude of pearl millet, white gram, black gram and white rice.

## Conclusion

Methanolic extract of barley, and Bengal gram exhibited significant antihyperglycemic activity along with antioxidant properties however, other antioxidant rich food grain extracts displayed pro-glycemic pattern. Complex kinetic pattern for

antioxidant activities in our study emphasize that the end point measurements for antioxidant activities of food products or chemical compounds would be appropriate only when the reaction has reached at least near to the completion specifically while explaining antioxidant capacity. Food grain extracts displayed linear as well as non-linear patterns in inhibiting AGEs formation. To the best of our knowledge this is the first report identifying pro-glycemic and antihyperglycemic properties in antioxidant rich fraction from food grains.

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