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ORIGINAL ARTICLE

Administration of Roasted Barley and Roasted Horse Gram Powders Pacified Chronic Sucrose-induced Dysglycemia and Dyslipidemia in Rats and Exerted *In Vitro* Potent Antioxidative Stress Effect

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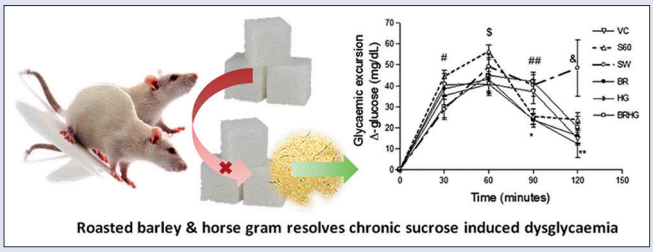
ABSTRACT

Background: Sugar-enriched diets/beverages consumption aggravates dysglycemia, dyslipidemia, insulin resistance, glucose intolerance, and weight gain. If unchecked, these disturbances culminate into diabetes. Indian medical classics advise consumption of roasted barley (BR) and roasted horse gram (HG) to resolve such issues. **Objective:** To investigate impact of BR and HG in chronic sucrose-induced dysglycemic rats. **Materials and Methods:** Dysglycemia was induced in rats by oral feeding of 40% sucrose solution continuously for 2 months. Later, rats were treated with test samples at a dose of 4 g/kg body weight twice a day for 1 month after withdrawal of sucrose feeding. Oral glucose tolerance test was performed at the end of experimental period. Biochemical and hematological parameters were analyzed accordingly. Nutritional contents and antioxidative stress activities in food grain powders were evaluated. **Results:** Chronic sucrose feeding induced glucose intolerance and weight gain. Mere sucrose withdrawal in the absence of supportive therapy aggravated glucose intolerance. Increase in plasma triglycerides, aspartate transaminase, and alanine transaminase and disturbances in hematological parameters were also observed. BR and HG treatment pacified sucrose-induced biochemical metabolic disturbances and improved hematological parameters. BR was superior in normalizing disturbances than horse gram. These grains possess potent antioxidant activities and antioxidative stress properties and are rich source of micronutrient vitamins, minerals, essential amino acids, and fatty acids. **Conclusion:** Consumption of these food grains may resolve and normalize sucrose-induced metabolic, biochemical, and hematological disturbances. Multiple therapeutic properties originating through phytochemicals, vitamins, minerals, amino acids, and fatty acids in totality may be responsible for observed beneficial effects.

Key words: Antioxidative stress, dysglycemia, dyslipidemia, glucose tolerance curve, nutritional composition, roasted barley and horse gram, sucrose

SUMMARY

- High sucrose consumption induces dysglycemia, dyslipidemia, and weight gain and disturbs hematological profiles
- Sudden sucrose withdrawal in the absence of supportive therapy aggravates dysglycemia
- Roasted barley and horse gram therapy reconciled chronic sucrose-induced dysglycemia and dyslipidemia in rats.



Roasted barley & horse gram resolves chronic sucrose induced dysglycaemia

Abbreviations used: ABTS^{+}: 2,2′-azinobis-(3-ethylbenzothiazoline -6-sulfonic acid) radical cation; AGEs: Advanced glycation end-products; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BR: Roasted barley; CHO: Chinese hamster ovary cells; DCFDA: 2′,7′-dichlorofluorescein diacetate; DPPH: 2,2-diphenyl-1-picrylhydrazyl; DMEM: Dulbecco's modified Eagle's medium; EDTA: Ethylenediaminetetraacetic acid; $FeCl_3$: Ferric chloride; HEK: Human embryonic kidney cells; HG: Roasted horse gram; H_2O_2: Hydrogen peroxide; MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NBT: Nitro-blue tetrazolium; OGTT: Oral glucose tolerance test; SW: Sucrose-withdrawn rats; S60: Sixty-day sucrose-fed rats; TC: Plasma total cholesterol; TG: Plasma triglyceride; VC: Vehicle control normal rats.

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INTRODUCTION

Diabetes is no longer a challenge for rich nations only. It has rapidly spread its tentacles globally. According to the recent WHO estimates, diabetes is increasing noticeably in middle-income countries.[1] Diabetic people meet shattering personal health issues because it adversely affects heart, blood vessels, eyes, kidney, and nervous system. These challenges negatively influence active phases of individual's life. Its prevalence has doubled since 1980, and unmanaged high blood glucose

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level is responsible for >43% of all deaths before the age of 70 years. Undiagnosed and untreated diabetes that is silently surviving in different populations is latently deteriorating health condition of people's active life. Although overweight and obesity are the strongest risk factors for type 2 diabetes development, control of blood glucose is a prime matter to prevent incidences and slowing progression of diabetic complication development.[1] According to the estimate of 2013, South-East Asian countries harbored >72 million diabetics and the prevalence was reported to increase sharply in urban as well as rural areas. The reason cited was lifestyle transition from traditional toward urbanization and industrialization.[2] In India, the prevalence of diabetes is heterogeneous. Large differences in diabetes' prevalence in different states of India are reported recently.[3] Reports also noticed speedy epidemiologic transition in low socioeconomic strata of society in economically developed states.[3] Furthermore, the rising risk of type 2 diabetes development at younger age globally,[4] urgently requires adoption of robust preventive measures and evolve strategies to design and develop diabetes care for people who find difficult the access of advanced medical facilities.

In fact, rapid urbanization and industrial growth in the developing nations have disrupted the traditional food pattern and physical labor-intensive lifestyle of people and brought into the place fast-food culture and sedentary lifestyle. Although fast-foods have facilitated and eased life of hectic modern lifestyle, they are highly processed calorie-rich, energy dense but lack micronutrients, fibers, and phytochemicals required to balance and maintain normal biochemical and physiological homeostasis. The trending fast-food culture, therefore, is distressing normal biochemical and physiological processes resulting in number of metabolic disorders, including dysglycemia and dyslipidemia. Therefore, rapidly increasing consumption of processed foods and beverages is being linked to accelerate increasing prevalence and incidence of noncommunicable diseases including type 2 diabetes mellitus and obesity.[5-8] These realizations have shifted attention of health researchers identify minimally processed foods that are less hyperglycemic and more satiating.[9] In this regard, urge is being made to adopt integrated holistic preventive measures where minimally processed food materials do not lose their natural food matrix and retain optimal nutrients.[10]

Traditional classics of Indian medicines provide number of minimally processed ready-to-eat food preparations for diabetic patients. Roasted horse gram (HG), Bengal gram, black gram, and pigeon pea powders have been prescribed in *Sushruta Samhita* as alternative fast-foods for diabetics.[11] In *Charak Samhita*, roasted barley (BR) powder is advocated for diabetic people.[12] It is mentioned that people consuming BR powder resist diabetes development.[12] For the management of diabetes, invigorated barley beverage has been prescribed.[13] In the states of India where the prevalence of diabetes has been recorded less,[3] these fast-foods are still order of the day. However, scientific basis for such folk-knowledge has hardly been evaluated.

Increased consumption of sugar-enriched diets and beverages has unequivocally been identified as major risk factor increasing global prevalence of diabetes mellitus and obesity.[7,14,15] In animal models, chronic sucrose-feeding has been demonstrated to cause dysglycemia and dyslipidemia and induce symptoms of prediabetes.[16,17] Furthermore, this animal model has also been utilized in delineating health benefits of dietary interventions in resolving issues of prediabetes.[18]

The aim of the present study was to scientifically evaluate traditional prescription that consumption of BR and HG powders prevents the development of diabetes and is a healthy dietary adjunct for diabetics. For that, rats were fed with sucrose solution daily for 2 months to induce dysglycemia and dyslipidemia and thereafter treated with BR and HG powders for 1 month. Because hyperglycemia is known to aggravate free radical generation and induce oxidative stress responsible for the development of diabetic complications;[19,20] antioxidative stress potentials (AoxPs) of preparations were also tested adopting *in vitro* cell-based assays. In addition, this study also presents in detail the nutrition compositions in these two food products. Furthermore, efforts were made to elucidate plausible mechanism of antihyperglycemic and antihyperlipidemic activities in BR and HG powders.

MATERIALS AND METHODS

Sample preparation

Barley (*Hordeum vulgare* Linn.) and horse gram (*Macrotyloma uniflorum* Lam.) healthy seeds were procured from local market in Hyderabad. Seeds were properly cleaned to remove any foreign material. Barley grains were soaked in water to swell and dried. Dried barley grains were pounded to remove husk and cleaned properly. Horse gram seeds were not given such treatment and were used with seed coat intact. Seeds were sea-sand roasted in bowl-shaped iron pan at approximately 280°C for maximum grain expansion avoiding burning.[21] Cooled grains were fine powdered and stored in air-tight containers for nutritional analysis and animal experiment. Extracts (50% aqueous methanol) of roasted seed powder was prepared for analysis of total polyphenols and flavonoids content as well as for determination of *in vitro* biological activities.

Animal experiments

Animal study was performed on Wistar rats (weight 200 ± 10 g body weight). All the experimental protocols were approved by the Institutional Ethical Committee (CPCSEA-97/GO/RBi/S/1999/CPCSEA, dated October 01, 2015) of the institute. The experiments were performed on live animals in compliance with the relevant laws and institutional guidelines and rats were treated with human care. Rats were initially examined for their general health and overnight-fasted rats' blood was collected from retro-orbital plexus in ethylenediaminetetraacetic acid containing tubes. Plasma glucose level was estimated using kit method on auto blood analyzer (Dimension Xpand Plus, Siemens; Health Care, Germany). Rats with normal level of plasma glucose level (70–90 mg/dL) were chosen for experiment. Rats were given twice a day 40% sucrose solution orally at the dose of 10 mL/kg body weight continuously for 2 months (60-day sucrose-fed rats [S60]). The dose and duration of sucrose feeding in rats to induce dysglycemia were decided based on our earlier studies.[18,22] A group of normal control rats (vehicle control [VC], $n = 6$) were maintained throughout the study period, and animals were treated sham. Rats received their normal pellet diet and water *ad libitum* throughout the study period. After 2 months sucrose feeding, oral glucose tolerance test (OGTT) was performed by administering 2 g/kg body weight glucose in overnight-fasted animals as reported earlier.[18] Sucrose feeding was withdrawn and rats were divided as the following four groups.

- Group-I: Sucrose-withdrawn control (SW): This group of rats received sham treatment throughout the study period of 1 month
- Group-II: Sucrose withdrawn BR powder treatment: To this group of rats was given BR powder treatment (4 g/kg body weight/day, in two divided dosage [2 g/kg body weight]) morning and evening for 1 month
- Group-III: Sucrose-withdrawn HG powder treatment: This group of rats received HG powder treatment (4 g/kg body weight/day, in two divided dosage [2 g/kg body weight]) morning and evening for 1 month
- Group-IV: Sucrose-withdrawn BR and HG powder mixture treatment (BRHG): This group of animals received BRHG power (in the ratio of 3:2) treatment (4 g/kg body weight/day, in two divided dosage [2 g/kg body weight]) morning and evening for 1 month. This ratio of mixing BR and HG was purely based on prevailing

folk-practices. The dose of BR, HG, and their mixture was decided based on our previous report.[23]

All the animals continued to receive normal pellet diet and water *ad libitum* throughout the experimental period.

At the end of the study period, OGTT was performed as stated earlier; blood samples were collected and used for the estimation of hematological and other biochemical parameters.

Plasma triglycerides (TG), total cholesterol (TC), aspartate transaminase (AST), and alanine transaminase (ALT) were estimated using kits provided by the manufacturer on auto blood analyzer (Siemens Health Care Ltd., Germany). Hematological parameters were analyzed using Advia 2120i Hematology Analyzer (Siemens Health Care Ltd., Germany).

In vitro biological activities

Antioxidant activity

Antioxidant activity was determined on multiple test systems. The 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^+$) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging potentials, nitro-blue tetrazolium (NBT), and ferric chloride reducing power as the measures of antioxidant activity were determined in aqueous methanol extract of roasted powders as detailed earlier.[24] Trolox and ascorbic acid, respectively, served as standard antioxidant reference compound.

Advanced glycation end-products inhibitory activity

AGEs screening was performed according to Poornima *et al.* (2016)[25] Aminoguanidine (5 mg/mL) was used as standard. Both vesperlysine-like (ʎexc 370 nm; ʎem 440 nm) and pentosidine-like (ʎexc 335 nm; ʎem 385 nm) AGE inhibition was determined according to Séro *et al.*,[26] using BioTek Synergy 4 Multimode Microplate Reader (BioTek Instruments Inc., Winooski, VT, USA). Results were expressed as percentage inhibition of AGEs.

Pancreatic α-amylase activity assay

Extracts were primarily incubated with enzyme and the mixtures were reacted with substrate soluble potato starch solution (0.5% w/v in 20 mM phosphate buffer, pH 6.9) for 3 min. DNS color reagent (1.0 g of 3,5-dinitrosalicylic acid, 30 g of sodium potassium tartarate, and 20 mL of 2 N NaOH to a final volume of 100 mL in distilled water) was added. Closed tubes were placed in a water bath (85°C–90°C) for 10 min to develop color and cooled thereafter. 50 µL of reaction mixtures was diluted with 175 µL of distilled water in a 96-well microplate. α-Amylase activity was determined by measuring the absorbance of the mixture at 540 nm spectrophotometrically[27] on BioTek Synergy 4 Multimode Microplate Reader (BioTek Instruments Inc., Winooski, VT, USA).

Rat intestinal α-glucosidase activity

Preincubated with extracts, the crude rat intestinal α-glucosidase enzyme was reacted with substrate p-nitrophenyl-α-D-glucopyranoside, and release of p-nitrophenyl due to action of α-glucosidase enzyme was recorded spectrophotometrically.[23]

Pancreatic lipase activity

Pancreatic lipase inhibition was determined according to the procedure described by McDougall *et al.*[28] Porcine pancreatic lipase type II (Sigma Aldrich) dissolved in MilliQ water was preincubated with extracts, and mixture was reacted with p-nitrophenyl laurate substrate. Enzyme activity was recorded measuring release of p-nitrophenyl spectrophotometrically at 405 nm.

Antioxidative stress potential in different cell lines

Human embryonic kidney cells (HEK)-293 and Chinese hamster ovary (CHO) cells were procured from the National Centre for Cell Sciences (Pune, India) and were grown in tissue culture flask in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% fetal bovine serum and 1X antibiotic solution (Sigma) in a CO_2 incubator at 37°C (5% CO_2 and 90% relative humidity).

Hydrogen peroxide-induced oxidative stress and effect of aqueous methanol extract on human embryonic kidney cells-293, and Chinese hamster ovary cells

The effect of aqueous methanol extract on hydrogen peroxide (H_2O_2)-induced oxidative stress[29] on cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[30] HEK-293 and CHO cells (1×10^6) were seeded in 96-well plates for 24 h. After 24 h of incubation, cells were treated with different concentrations of extract for 48 h in the presence and absence of 10 µM H_2O_2.[29] The concentration of extract was randomly selected between 0.1 µg and 10 µg as working concentration in culture medium. After 48 h of incubation, 10 µl MTT (Sigma, 5 mg/mL) was added to each well and plates were further incubated for 4 h at 37°C in the dark. Culture medium from each well was carefully removed out and 100 µl of dimethyl sulfoxide was added. Reduction MTT by metabolically viable cell was determined measuring absorbance at 570 nm spectrophotometrically.

AoxP of test extract was calculated as follows:

$100 - [\{(A_c - A_t)/Ac\} \times 100]$, where "$A_c$" represent absorbance of cells without H_2O_2 treatment and "A_t" represent absorbance of cells treated with H_2O_2 in the presence or absence of extract.

Nutritional and phytochemical analysis

Nutritional analysis of BR and HG powder was carried out at MFPI-Quality Control Laboratory of Professor Jayashankar Telangana State Agricultural University, Hyderabad (India).

Proximate analysis

Analysis of carbohydrate,[31] protein,[32] fat,[32] fiber,[32] ash,[33] and moisture[33] contents was done based on the following standard methods. The acidity value was determined following IS 13844:2003 Methodology.[34]

Sugars

Total as well as reducing sugars present in BR and HG powders were analyzed spectrophotometrically.[35]

Vitamins

Vitamins such as riboflavin, thiamine, and niacin were quantified by high-performance liquid chromatography method as reported by Anyakora *et al.*[36] Ascorbic content was titrated following method described in Ranganna.[37]

Amino acids

Analysis of amino acids was carried out by high-performance liquid chromatography applying photodiode array (diode array detector [DAD]) and fluorescence detection simultaneously (DAD: 338 10 nm; Ref 390 20 nm, Fluorescence: Ex 340 nm, Em 450 nm) as described by Steed.[38]

Fatty acids

Gas chromatographic analysis of fatty acid methyl esters (FAMEs) was performed, and relative retention time of FAME peaks was compared with standard samples of fatty acid for their identification.

Minerals

Inductively coupled plasma optical emission spectrometry technique was used to measure iron, zinc, and calcium content in BR and HG powders.

Phytochemicals

Total polyphenol

Total polyphenol content in aqueous methanol extract was measured using Folin–Ciocalteu reagent as mentioned earlier.[39] Absorbance at 765 nm was recorded spectrophotometrically and the results were expressed in terms of gallic acid equivalence.

Total flavonoid

Equal volume of 2% aluminum chloride was mixed with aqueous methanol extract of grain's powder to quantify flavonoids content.[39] Absorbance was recorded at 430 nm spectrophotometrically and the results were expressed in terms of rutin equivalence.

Phytate

Phytate was extracted from powders with trichloroacetic acid and further precipitated with ferric salt. The iron content of precipitate was determined colorimetrically, and phytate content was determined as described by Wheeler and Ferrel.[40]

Oxalate

Total oxalate was extracted with 0–25 N hydrochloric acid. Titration method using permanganate was applied for the determination of oxalate as described by Sanchez-Alonso and Lachica.[41]

Total carotenoids

Spectrophotometric techniques described by Zakaria *et al.*[42] were adopted for the determination of total carotenoids in roasted seed grain's powders.

Lycopene

Lycopene content was estimated as described by Ranganna.[37] Lycopene content in roasted seed grain's powders was repeatedly extracted with acetone followed by petroleum ether. Lycopene color was measured at 503 nm spectrophotometrically. Percentage content was calculated as per the formula provided by authors.

Statistical analysis

The results were analyzed using *t*-test with Welch's correction and one-way analysis of variance followed by Tukey's multiple comparison test as applicable. Criterion for statistical significance was set at $P < 0.05$. Statistical analysis was performed by using GraphPad PRISM Version 5.01 (GraphPad Software Inc., CA, USA).

RESULTS

Glycemic excursion following oral glucose tolerance test

The glycemic excursion following OGTT at different time points and total glycemic excursion up to 2 h in different groups of rats is presented in Figure 1. Up to 30 min, differences between glycemic excursion in VC normal rats and S60 were not different. However, rapid increase was noticed after 30 min, and the glycemic excursion in S60 rats was significantly ($P < 0.04$) higher than VC rats [Figure 1a]. Thereafter, differences in incremental increase in glucose levels receded in both the groups and differences were not significant up to 120 min. The rise in plasma glucose level following OGTT in SW group was initially slower than the rise in plasma glucose level of S60 rats. Differences were significantly less ($P < 0.02$) at the 30th min. At the 60th min, however, values of glycemic excursion were close to each other [Figure 1a]. However, after 60 min, glycemic excursion in S60 rats decreased and matched VC normal rats; it was still significantly high ($P < 0.039$) in SW group rats from the values of S60 rats at 60 min and did not decrease even at 120 min. However, differences were not statistically significant ($P = 0.128$) at this time point. It is noteworthy to mention here that the fluctuations in glucose level at 120 min in SW rats were more than that observed in S60 rats [Figure 1a].

The changes in glycemic excursion in rats receiving BR, HG, and BRHG powders followed the glycemic curve pattern of VC rats following OGTT [Figure 1a]. At 90 min, incremental glucose values in BR- and HG-treated rats were significantly ($P < 0.025$) less than SW rats [Figure 1a]. At 120 min also, incremental value in HG-treated rats was significantly ($P < 0.046$) less than SW rats. However, even after being less than SW group, differences between BR-treated rats and SW rats could not reach degree of statistical significance ($P = 0.07$) at 120 min time point. Glycemic excursion up to 120 min in terms of absolute Δ-glucose in BR powder and BRHG mixture-treated rats were significantly ($P < 0.008$ and $P < 0.04$, respectively) less than SW group rats [Figure 1b]. Although this value was less in HG-treated rats also, the statistically differences were not significant ($P = 0.057$) than SW rats. The absolute Δ-glucose value in SW rats was 32% higher than values in normal VC rats; however, differences were not statistically significant [$P = 0.07$, Figure 1b].

Changes in weight following sucrose feeding and different treatments

Changes in weight of rats receiving sucrose solution were weekly monitored up to 60 days and also during the treatment period. Results are presented in Figure 2. Significantly more (8 g, $P < 0.037$) increase in weight of rats receiving sucrose was noticed on the 14th day and difference reached up to 32 g on the 60th day ($P < 0.0016$) when compared with the weight gain trend in normal VC control rats [Figure 2a]. Following 30-day treatments, although the weight reduction in HG powder receiving animals was observed, it was not significantly different than the change in weight of VC normal rats [Figure 2b]. Slight gain in the weight of rats receiving BR and BRHG was observed after 30 days; here also, the differences were not statistically significant when compared with normal VC rats or the SW group rats [Figure 2b].

Changes in plasma triglycerides and total cholesterol

TG and TC levels were estimated after 30 days treatments in respective group of rats and values are presented in Figure 3. TG values were high (29%) in SW rats receiving only normal laboratory chow diet and water *ad libitum* for 30 days than normal VC rats. However, differences in values were not statistically significant ($P = 0.20$). On the other hand, TG values in rats receiving BR powder and BRHG mixture were significantly ($P < 0.03$) less than the TG value of SW rats [Figure 3a]. TG value was also less in HG-treated rats, but differences were not statistically significant when compared with SW rats [Figure 3a]. Differences in triglyceride values in the treatment groups were not statistically different than triglycerides level of vehicle control normal rats [Figure 3a]. The TC levels were not different in treatment groups except in rats receiving BRHG mixture when compared with SW group of rats [Figure 3b, $P < 0.002$]. When compared with normal VC rats, TC levels were statistically not different in any treatment group [Figure 3b].

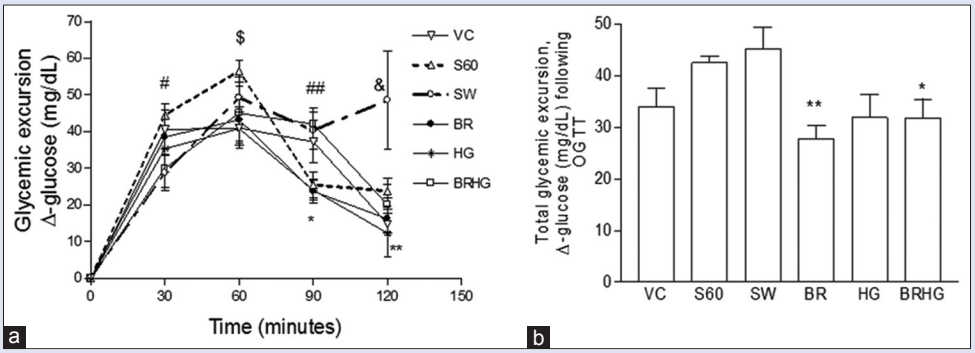

Figure 1: Glycemic excursion following oral glucose tolerance test. (a) Incremental increase in plasma Δ-glucose level from basal level at different time points. Unpaired *t*-test with Welch's correction was applied to test differences between groups. $^{\#}P < 0.02$ (S60 vs. SW), $^{\$}P < 0.04$ (S60 vs. VC), $^{\#\#}P < 0.03$ (S60 vs. SW), and $P = 0.12$ (S60 vs. SW), $^{*}P < 0.02$ (SW vs. BR and HG), $^{**}P < 0.04$ (SW vs. HG). (b) Total glycemic excursion load up to 2 h. Unpaired *t*-test with Welch's correction was applied to test differences between groups. $^{**}P < 0.008$ (SW vs. BR), $^{*}P < 0.04$ (SW vs. BRHG). Data represents mean ± standard error of mean, $n = 40$ in case of S60 and $n = 6$ in all other groups. SW: Sucrose-withdrawn rats; S60: Sixty-day sucrose-fed rats; BR: Roasted barley; VC: Vehicle control normal rats; HG: Roasted horse gram; BRHG: Mixture of roasted barley and roasted horse gram

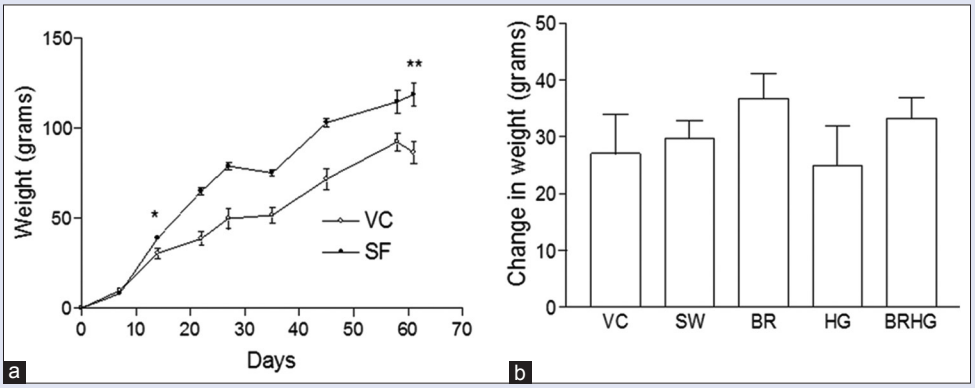

Figure 2: Change in weight of rats due to sucrose feeding and treatment with BR and HG powders and BRHG. (a) Weight gained by animals during SF period. Unpaired *t*-test with Welch's correction was applied to test differences between the groups. $^{*}P < 0.03$ (VC vs. SF), $^{**}P < 0.001$ (VC vs. SF). Data represent mean ± standard error mean, $n = 40$ in case of SF and $n = 6$ in VC group. (b) Change in weight of rats following sucrose withdrawal and start of treatment for up to 30 days. Weight differences were not significantly different among compared groups. SW: Sucrose-withdrawn rats; BR: Roasted barley; VC: Vehicle control normal rats; HG: Roasted horse gram; BRHG: Mixture of roasted barley and roasted horse gram; SF: Sucrose feeding rats

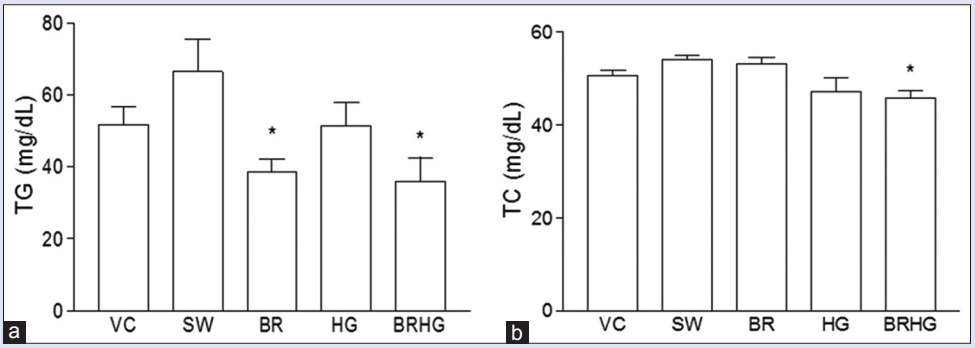

Figure 3: TG and TC levels in different treatment groups. (a) TG level in different treatment groups. Unpaired *t*-test with Welch's correction was applied to test differences between groups. $^{*}P < 0.03$ (SW vs. BR and BRHG). (b) TC levels in rats receiving different treatments. Unpaired *t*-test with Welch's correction was applied to test differences between groups. $^{*}P < 0.002$ (SW vs. BRHG). Values represent mean ± standard error of mean, $n = 6$. SW: Sucrose-withdrawn rats; BR: Roasted barley; VC: Vehicle control normal rats; HG: Roasted horse gram; BRHG: Mixture of roasted barley and roasted horse gram; TG: Plasma triglycerides; TC: Plasma total cholesterol

Changes in liver enzymes' aspartate aminotransferase and alanine aminotransferase

Liver enzymes in different treatment groups are presented in Figure 4. It was observed that AST values were significantly (2.4 times, $P < 0.01$) high in SW rats than AST levels in normal VC rats [Figure 4a]. AST values were close to normal VC rats when SW rats were treated with BR, HG, and HGBR mixture and were significantly ($P < 0.05$) less than AST values of SW rats [Figure 4a]. Although the AST levels in HG-treated rats were less than SW rats, the difference was not statistically significant.

The ALT values in rats receiving in BR, HG, and BRHG mixture were significantly ($P < 0.05$) less when compared with ALT values of SW rats [Figure 4b]. The ALT values in SW group of rats were 3 times higher ($P < 0.05$) than ALT values of normal VC rats [Figure 4b]. Although the ALT values in rats receiving BR, HG, and BRHG mixture appear higher than VC normal rats, differences were not statistically significant [Figure 4b].

Hematological observations

Hematological parameters were analyzed in rats of different groups before start of the treatment and compared with values obtained after 30 days of treatment [Table 1].

Leukocytes count did not differ from their initial values in SW, BR, HG, and BRHG mixture treated rats after 30 days; however, it was found significantly ($P < 0.0014$) decreased in normal VC rats [Table 1].

Platelets count was found significantly ($P < 0.0199$) increased after 30 days treatment in rats receiving HG powder from their initial levels. Changes in platelet count in other groups did not differ significantly from their basal levels [Table 1].

Neutrophils count in normal VC rats and SW rats were found significantly ($P < 0.0001$ and $P < 0.035$, respectively) increased after 30 days with no significant change in other group of rats [Table 1].

Lymphocytes count did not statistically differ from their initial levels after 30 days in BR and HG powders treated rats. However, significant decline was noticed in normal VC rats ($P < 0.007$), SW rats ($P < 0.0.0003$), and rats who received BRHG mixture [$P < 0.0003$, Table 1].

Increase in monocytes count was noticed in SW group ($P < 0.0001$), BR powder ($P < 0.0.0006$), HG ($P < 0.0001$), and rats receiving BRHG mixture ($P < 0.03$) except in normal VC rats after 30 days [Table 1]. Eosinophils count did not differ statistically in any group from their initial counts after 30 days [Table 1].

Hemoglobin level was found significantly improved with 30 days treatment with BR powder ($P < 0.0017$), HG powder ($P < 0.0002$), and BRHG mixture ($P < 0.0135$). However, no change in hemoglobin level was observed in SW rats and normal VC rats from their initial values [Table 1].

Phytochemical analysis, free radicals scavenging antioxidant, and enzyme inhibitory activities

Table 2 presents the analysis of phytochemicals, free radicals scavenging antioxidant, and enzyme inhibitory activities in 50% aqueous methanol extract of BR and HG powder. Total polyphenols were five times ($P < 0.0001$) more in BR extract than present in HG extract. Similarly, flavonoids content in the BR extract was 2.5 times ($P < 0.0006$) higher than that present in HG extract [Table 2].

The $ABTS^+$ scavenging (SC_{50} = 3.8 μg/mL) and NBT reducing potential (RC_{50} = 3.3 μg/mL) of HG extract were double than that present in BR extract. However, DPPH radical scavenging activity in BR extract was twice (SC_{50} = 5.9 μg/mL) than that of the HG extract [SC_{50} = 13.0 μg/mL, Table 2]. The presence of ferric reducing power in HG extract was twenty times more than the ferric reducing power present in the BR extract.

The inhibitory activity of v-type AGEs formation in HG was four times ($P < 0.0002$) more than that present in the BR extract [Table 2]. However, formation of p-type AGEs was inhibited more ($P < 0.01$) by BR extract.

Pancreatic α-amylases were inhibited more potently ($P < 0.007$) by HG extract than extract of BR. The pancreatic α-amylases inhibitory activity in HG extract was comparable to the standard drug acarbose [Table 2]. Similarly, the presence of intestinal α-glucosidase inhibitory activity in HG extract was significantly ($P < 0.001$) higher than inhibitory activity present in the extract of BR [Table 2]. Here too, the intestinal α-glucosidase inhibitory activity present in the HG extract was close to the inhibitory activity of standard drug acarbose.

The pancreatic lipases were inhibited more potently ($P < 0.0001$) by BR extract than HG extract [Table 2].

Antioxidative stress activity on cell lines

Influence of different concentrations (0.1, 1.0, and 10.0 μg) of 50% aqueous methanol extract of BR and HG powder on H_2O_2-induced oxidative stress in normal HEK-293 and CHO cells is presented in Figure 5. Significant ($P < 0.01$) decrease in HEK-293 cells viability was noticed when cells were incubated with H_2O_2 compared with

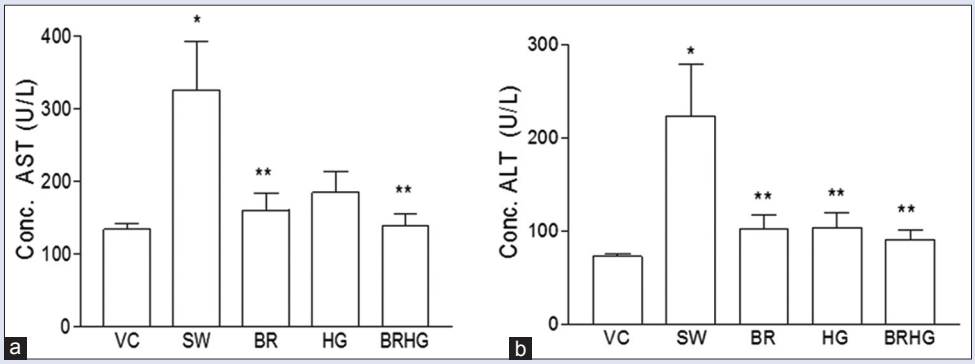

Figure 4: Liver function test of rats under different treatments. (a) Plasma AST levels in different treatment groups. *$P < 0.01$ (SW vs. VC), **$P < 0.05$ (SW vs. BR and BRHG). (b) Plasma ALT levels in different groups. *$P < 0.01$ (SW vs. VC), **$P < 0.05$ (SW vs. BR, HG and BRHG). analysis of variance followed by Tukey's multiple comparison tests was applied to analyze differences in this test. Values represent mean ± standard error of mean, $n = 6$. SW: Sucrose-withdrawn rats; BR: Roasted barley; VC: Vehicle control normal rats; HG: Roasted horse gram; BRHG: Mixture of roasted barley and roasted horse gram; SF: Sucrose feeding rats; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase

Table 1: Hematological examination

Groups	Leukocytes (×10³)		Erythrocytes (×10⁶)		Hemoglobin (g/dL)		Platelets (×10³)		Neutrophils (%)		Lymphocytes (%)		Monocytes (%)		Eosinophils (%)	
	0 day	30th day	0 day	30th day	0 day	30th day	0 day	30th day	0 day	30th day	0 day	30th day	0 day	30th day	0 day	30th day
VC	9.73±0.70	5.85[a]±0.41	8.75±1.18	7.45±0.15	13.76±0.26	13.7±0.23	982.80±42.93	970.75±81.8	15.10±0.92	22.65[f]±0.80	73.36±1.85	65.00[h]±0.45	4.60±0.53	4.22±0.30	5.02±0.61	6.52±0.45
SW	7.78±0.17	6.99±0.77	7.97±0.46	7.77±0.11	13.36±0.24	13.80±0.26	837.00±52.60	932.80±81.8	16.1±0.92	22.22[g]±2.07	71.32±1.85	56.06[i]±1.97	4.38±0.53	8.70[k]±4.10	6.66±1.55	9.06±0.52
BR	6.76±0.45	6.68±0.14	7.68±1.10	7.92±0.07	13.08±0.18	14.12[b]±0.07	828.0±32.86	793.0±27.67	18.02±0.92	17.00±2.62	70.44±1.85	66.37±7.30	3.60±0.53	7.30±0.53	6.32±1.55	5.35±0.58
HG	8.75±0.77	7.52±0.82	7.25±0.18	7.35±0.08	11.96±0.24	13.94[e]±0.22	849.00±44.2	1034.0[e]±48.3	24.82±0.92	22.96±0.92	63.84±1.85	59.48±1.56	2.82±0.26	5.90[m]±0.26	7.06±0.92	8.36±0.92
BRHG	9.73±0.70	7.30±0.91	8.75±0.18	7.78±0.23	12.36±0.37	13.82[d]±0.30	982.00±42.9	909.0±30.32	24.62±2.03	29.80±2.28	64.12±1.25	54.35[j]±1.16	3.24±0.48	4.80[n]±0.38	6.56±1.45	9.60±0.61

Unpaired *t*-test with Welch's correction (two-tailed). [a]$P<0.0014$, [b]$P<0.0017$, [c]$P<0.0002$, [d]$P<0.0135$, [e]$P<0.0199$, [f,k,m]$P<0.0001$, [g]$P<0.035$, [h]$P<0.007$, [i,j]$P<0.0003$, [l]$P<0.0006$, [n]$P<0.03$ when compared with respective 0 day values (n=5). NS: Not significant; SW: Sucrose withdrawn; HG: Roasted horse gram; BRHG: Roasted barley and roasted horse gram; VC: Vehicle control normal rats; BR: Roasted barley

HEK-293 cells without any treatment [Figure 5a]. Preincubation of cells with different concentrations of extracts mitigated H_2O_2-induced oxidative stress. Protection against H_2O_2-induced oxidative stress was significantly ($P < 0.01$) high when cells were incubated with 0.1 and 1.0 µg of HG extract when compared with H_2O_2-treated cells [Figure 5a]. CHO cells viability was also found significantly ($P < 0.01$) decreased due to H_2O_2 treatment when compared with the viable CHO cells without any treatment [Figure 5b]. However, pretreatment of cells with different concentrations of 50% aqueous methanol extract of BR and HG powder prevented deleterious effect of H_2O_2 on cells and improved cell viability. Cell viability was significantly better when cells were preincubated with 1.0 µg ($P < 0.05$) and 10.0 µg of barley extract [Figure 5b]. Extract of HG at 0.1 and 10.0 µg also significantly ($P < 0.05$) prevented CHO cells against H_2O_2-induced oxidative stress [Figure 5b].

Nutritional profiles

The proximate compositions, sugars, vitamins, minerals, and phytochemicals present in the powder of BR and HG are presented in Table 3. Carbohydrate content in BR powder was 13% higher than carbohydrate content in HG. The protein content in HG was nonetheless double than that present in BR [Table 3]. Crude fiber and moisture content were found similar in both though ash content was high in HG powder. Total sugars and reducing sugar were recorded slightly higher in BR powder than HG powder although acidity value was measured high in HG powder [Table 3].

Vitamins content also varied in these two roasted grains [Table 3]. Per 100 g ascorbic acid content was 4 mg high in horse gram, whereas thiamin content was 184 µg more. Riboflavin content in BR powder was recorded 955 µg/100 g more and niacin content was 600 µg/100 g higher than that present in HG powder [Table 3].

Iron content per gram in HG was 2.5 mg more, whereas calcium content per 100 g in barley was 37 mg higher than present in HG [Table 3]. Zinc content was recorded 1470 µg/100 g more than barley in HG powder [Table 3].

Measurement of total carotenoids was in BR powder was 1761 µg/100 g more than total carotenoids measured in HG powder. Oxalate content was same in both the roasted grains, whereas phytate content in BR was 118 mg/100 g more than that present in HG powder. Microgram/100 g lycopene content in HG powder was 3 times more than BR powder [Table 3].

Amino acid profiles

Seventeen amino acids could be identified in BR and HG powder [Figure 6]. Amino acids are tabulated [Table 4] according to their retention time and respective peak elution. In terms of area percentage, glutamate, serine, histidine, alanine, tyrosine, and isoleucine elution was more or less equal. Percentage area elution of leucine was three times more in BR powder compared with HG. Cystine, phenylalanine, and aspartate elution were two times higher than in HG powder. On the other hand, percentage area elution of glycine, valine, and arginine in HG was more than twice than the percentage area elution of these amino acids in BR powder [Figure 6].

Fatty acid profiles

The gas chromatogram and percentage area elution of fatty acids in both the BR and HG powders are presented in Figure 7 and Table 5. Palmitic acid, stearic acid, oleic acid, linoleic acid, α-linolenic acid, and docosadienoic acid were present in both powders in varied amounts. Behenic acid was identified in HG powder [Figure 7].

Table 2: Phytochemical analysis, free radicals scavenging antioxidant potentials, and carbohydrate and lipid digesting enzyme inhibitory activities in 50% aqueous methanol extract of roasted barley and horse gram powder

Samples	Polyphenols (GAE, µg/mL)	Flavonoids (RE, µg/mL)	ABTS percentage scavenging (SC_{50}, µg/mL)	DPPH percentage scavenging (SC_{50}, µg/mL)	NBT percentage Reduction (RC_{50}, µg/mL)	$FeCl_3$ percentage Reduction (RC_{50}, µg/mL)	AGEs (percentage inhibition)		α-amylase (percentage inhibition)	α-glucosidase (percentage inhibition)	Pancreatic lipase (percentage inhibition)
							v-type	p-type			
BR	151.5±1.6[e]	50.1±1.4[c]	93.7±0.1 (8.9)	69.1±2.1 (5.9)	99.5±0.1 (6.2)	64.9±0.7 (0.23)	23.9±1.7	47.0±0.5[a]	20.9±0.3	29.0±0.6	44.2±0.5[e]
HG	32.47±0.3	20.5±1.3	95.1±1.0 (3.8)	72.7±3.4 (13.0)	95.8±0.3 (3.3)	61.2±1.4 (0.01)	84.9±2.1[d]	42.5±0.6	37.3±1.4[b]	57.6±0.8[e]	23.8±0.3
Trolox	-	-	98.2±0.9 (7.2)	-	-	-	-	-	-	-	-
Ascorbic acid	-	-	-	92.4±0.3 (3.1)	99.5±0.1 (2)	95.8±0.1 (0.03)	-	-	-	-	-
Aminoguanidine	-	-	-	-	-	-	74.5±0.2	90.0±2.4	-	-	-
Acarbose	-	-	-	-	-	-	-	-	37.9±1.8	64.4±1.2	-
Orlistat	-	-	-	-	-	-	-	-	-	-	54.3±4.6

Percentage activity was estimated with 1 mg/mL stock solution. Unpaired *t*-test with Welch's correction was applied to find differences between BR and roasted HG powder. Values in parentheses are SC_{50} or RC_{50}. [a]$P<0.01$, [b]$P<0.007$, [c]$P<0.0006$, [d]$P<0.0002$, [e]$P<0.0001$ when compared with their respective group. Values represent mean±SEM ($n\geq3$). GAE: Gallic acid equivalent; RE: Rutin equivalent, values in parentheses represent, SC_{50}: Radical scavenging concentration 50%; RC_{50}: Reducing concentration 50%; HG: Roasted horse gram; SEM: Standard error of mean; NBT: Nitro-blue tetrazolium; ABTS: 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl; $FeCl_3$: Ferric chloride; AGEs: Advanced glycation end products; BR: Roasted barley

Table 3: Nutritional profile of roasted barley and horse gram powders

	Roasted barley powder (%)	Roasted horse gram powder (%)
Proximate		
Carbohydrate	78.38	65.75
Crude protein	12.88	24.32
Crude fiber	4.36	4.36
Ash	1.87	3.79
Moisture	5.26	5.18
Sugars		
Total sugars	10.88 g/100 g	7.93 g/100 g
Reducing sugar	0.44 g/100 g	0.31 g/100 g
Acidity	0.70	1.72
Vitamins		
Ascorbic acid	2.24 mg/100 g	6.27 mg/100 g
Riboflavin	1.18 mg/100 g	0.225 mg/100 g
Thiamine	0.029 mg/100 g	0.213 mg/100 g
Niacin	1.4 mg/100 g	0.8 mg/100 g
Minerals		
Iron	4.74 mg/100 g	6.27 mg/100 g
Zinc	2.32 mg/100 g	3.79 mg/100 g
Calcium	120.64 mg/100 g	83.62 mg/100 g
Phytochemicals		
Total carotenoids	1924.07 µg/100 g	162.78 mg/100 g
Lycopene	0.0019 µg/100 g	0.0061 µg/100 g
Phytate	269.16 mg/100 g	151.12 mg/100 g
Oxalate	21.15 mg/100 g	20.88 mg/100 g

DISCUSSION

There are convincing evidence supporting link between consumption of sugary drinks and development of obesity and diabetes.[43] High-sucrose diet has been reported to induce dysglycemia, dyslipidemia, insulin resistance, and reduced glucose tolerance in rats.[16,44] High-sucrose feeding in rats has also been utilized to identify potential antidysglycemic natural therapeutics.[18]

In this experiment, 4.0 g/kg dose of sucrose dissolved in water (Milli-Q) was given orally to rats twice a day continuously for 2-month period in the morning and evening time. These timings were chosen to match human habits of morning breakfast and evening snacks time. We used incremental changes in glucose level over time following OGTT to carve out shape of glucose tolerance curve. The incremental increase in plasma glucose level in sucrose-fed rats (S60) was more between 30 and 60 min than the incremental increase in plasma glucose level of normal VC rats. However, deceleration in incremental glucose increase was noticed post 60 min in S60 rats and the shape of glucose tolerance curve resolved as that in the case of normal VC rats. Despite the resolution in shape of glucose tolerance curve in S60 rats, the weight of these rats was significantly higher than normal VC rats. Similar observations were reported very recently by Kowalski *et al.*[17] Authors opined that build-up of subcutaneous adipose tissues due to sucrose feeding augmented glucose disposal capacity in high-sucrose-fed mice despite the presence of insulin resistance. Augmentations in glucose disposal capacity in mice due to subcutaneous adipose tissue deposition in fact led glucose tolerance curve appear as normal.[17]

Glucose intolerance was found aggravated in rats after 1 month sucrose withdrawal even though the weight gain was compromised after sucrose feeding was stopped. Examination of shape of glucose tolerance curve showed that plasma glucose level did not decrease appreciably after 60 min and remained high up to 120 min. Such shape describes development of impaired glucose tolerance and poor insulin sensitivity which marks pathophysiological condition of type 2 diabetes.[45-47] These findings state that mere abstinence from sucrose

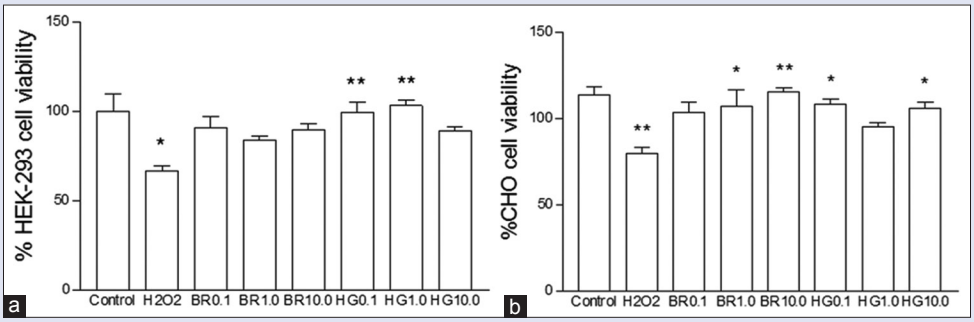

Figure 5: H_2O_2-induced oxidative stress and effect of BR and HG powder's 50% aqueous methanol extract on normal cell lines. (a) Effect of H_2O_2-induced oxidative stress on HEK-293 cells and influence of BR and HG powder's 50% aqueous methanol extract. *$P < 0.01$ (H_2O_2 vs. control), **$P < 0.01$ when compared with H_2O_2. (b) Effect of H_2O_2-induced oxidative stress on CHO cells and influence of roasted barley and horse gram powder's 50% aqueous methanol extract. *$P < 0.05$ when compared with H_2O_2. Suffix 0.1, 1.0, and 10.0 at BR and HG are µg concentrations of test samples. One-way analysis of variance followed by Tukey's multiple comparison tests was applied to test degree of significant differences. Values represent mean ± standard error of mean, $n = 3$. BR: Roasted barley; HG: Roasted horse gram; HEK: Human embryonic kidney cells; H_2O_2: Hydrogen peroxide; CHO: Chinese hamster ovary cells

Table 4: High-performance liquid chromatography data of amino acids present in roasted barley and roasted horse gram powders

DAD: Signal A, 338 nm/BW: 10 nm reference 390 nm/BW: 20 nm results		Roasted barley powder			Roasted horse gram powder		
Peak number	Name	Retention time	Area	Area percentage	Retention time	Area	Area percentage
1	Aspartate	2.782	37,340	6.02	2.832	11,919	3.64
2	Glutamate	5.292	212,592	34.25	5.018	121,857	37.24
3	Serine	7.857	39,382	6.34	7.915	18,876	5.77
4	Histidine	8.808	2243	0.36	8.875	1392	0.43
5	Glycine	9.112	37,489	6.04	9.170	38,232	11.68
6	Threonine	9.272	28,423	4.58	9.292	16,186	4.95
7	Arginine	9.507	2170	0.35	9.533	3154	0.96
8	Alanine	10.053	21,733	3.50	10.93	16,075	4.91
9	Tyrosine	10.540	41,236	6.64	10.590	17,533	5.36
10	Cystine	11.768	15,349	2.47	11.820	3041	0.93
11	Valine	13.172	7346	1.18	13.037	19,781	6.05
12	Methionine	13.858	31,778	5.12	13.845	10,674	3.26
13	Phenylalanine	14.010	12,284	1.98	14.035	2340	0.72
14	Isoleucine	15.400	24,015	3.87	15.335	9559	2.92
15	Leucine	15.585	37,145	5.98	15.525	6050	1.88
16	Lysine	16.232	67,902	10.94	16.163	24,811	7.58
17	Proline	17.507	2280	0.37	17.537	5647	1.73

DAD: Diode-array detector; HPLC: High-performance liquid chromatography data

Table 5: Gas chromatographic data of fatty acids in roasted barley and roasted horse gram powders

Back signal results	Roasted barley powder				Roasted horse gram powder			
Peak number	Name	Retention time	Area	Area percentage	Name	Retention time	Area	Area percentage
1	Palmitic acid methyl ester (C16:0)	25.852	388325	19.03	Palmitic acid methyl ester (C16:0)	25.858	594,937	22.69
2	Stearic acid methyl ester (C18.0)	30.247	23896	1.17	Stearic acid methyl ester (C18.0)	30.243	80,649	3.05
3	Oleic acid methyl ester (C18:1n9c)	30.654	316499	15.51	Oleic acid methyl ester (C18:1n9c)	30.655	432,228	16.49
4	Linoleic acid methyl ester (C18:2n6c)	31.640	1059481	51.93	Linoleic acid methyl ester (C18:2n6n)	31.641	1,054,398	40.21
5	α-Linoleic acid methyl ester (C18:3n3)	32.975	119621	5.86	α-Linoleic acid methyl ester (C18:3n3)	32.977	355,577	13.56
6	Cis-13,16-docosadienoic acid methyl ester	39.335	132271	6.48	Behenic acid methyl ester (c22:0)	38.088	60790	2.32
7					Cis-13,16-docosadienoic acid methyl ester	39.334	43943	1.68
Total			2,040,093	100.00			2,622,022	100.00

consumption may not help improve dysglycemic conditions once induced. Elicitation of other symptoms due to sucrose withdrawal[48,49] in dysglycemic condition may turn deleterious contributing to obesity and diabetes in the absence of adjunct therapeutic measures. Therefore, actions that prevent the development of sucrose-withdrawal symptoms appear important.

Glycemic response following OGTT in SW rats given BR, HG, and BRHG mixture was observed normalized. The aqueous methanol

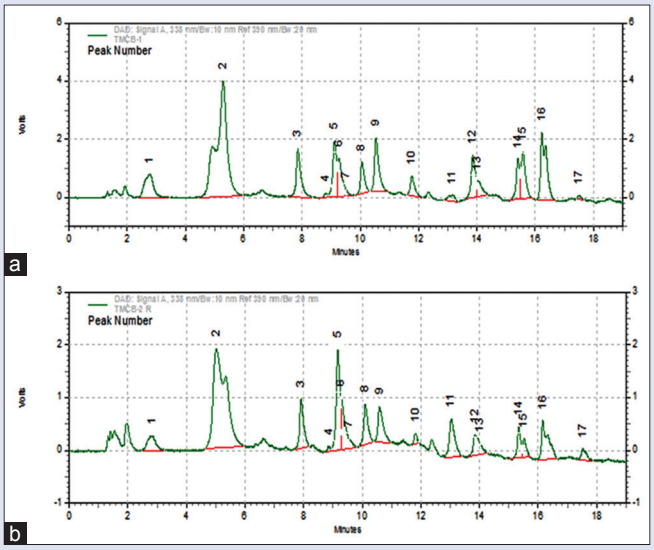

Figure 6: High-performance liquid chromatography chromatogram of amino acids (a) roasted barley and (b) roasted horse gram

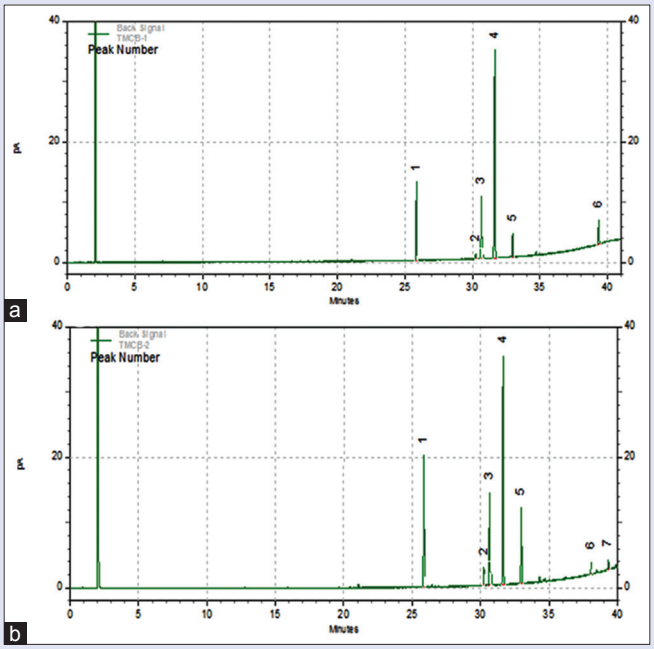

Figure 7: Gas chromatographic profiles of fatty acids (a) in roasted barley and (b) roasted horse gram powder

extract of these preparations possess pancreatic α-amylase and intestinal α-glucosidase inhibitory activities. Inhibitors of pancreatic α-amylase and intestinal α-glucosidase inhibitors have proved their clinical efficacy in controlling postprandial hyperglycemic excursions, and weight gain with very good conformity in type 2 diabetes patients.[50] Presence of these activities in BR and HG might be held responsible in part in resolving postprandial hyperglycemic disturbances caused due to sucrose feeding and its withdrawal in our study. BR followed by BRHG mixture was more effective in controlling absolute glycemic excursion. Although weight gain in rats was controlled following sucrose withdrawal and start of therapy, HG feeding appreciably controlled gain in weight of rats than BR and BRHG mixture. Whole barley kernel-based foods are now finding inclusion in dietary diabetes therapy.[51,52] BR and HG were prescribed in ancient Indian medical classics as fast-food item beneficial for diabetics. In practice, such foods constitute breakfast and snacks items. The timing of morning and evening for their feeding to experimental animals was chosen based on such prevailing practices in public.

Sucrose feeding in rats has been demonstrated to induce dyslipidemia hypertriglyceridemia in particular and worsen insulin secretion and insulin sensitivity.[44] In our study, TG level in SW rats was moderately higher than the TG level in normal VC rats. However, treatment of rats with BR, HG, and BRHG normalized triglycerides level after sucrose withdrawal. Efficacy of BR was superior in reducing triglycerides level in rats than powder of HG. Such anti-hypertriglyceridemic activity might arise in part due to the presence of pancreatic lipase inhibitory activities in the extract of these food grains. TC level was not affected in our study in any group; however; level of TC was less in rats treated with BRHG mixture when compared with the TC level in SW rats.

High carbohydrate particularly sucrose-rich high-calorie diet is known to increase blood level of transaminases and triglycerides in healthy male volunteers.[53] Increased level of ALT was frequently noted by Everhart[54] in diabetic population also. Increased levels of ALT and AST are markers of liver malfunction. The values of these enzymes were high in SW control rats however; levels of these enzymes were normalized when SW rats received treatment of BR, HG, and BRHG.

Changes in hematological parameters in newly diagnosed cases of diabetes have been reported recently by Alam *et al.*[55] Authors reported significant increase in lymphocytes and monocytes count and decrease in neutrophils and monocytes counts along with significant decrease in hemoglobin levels than nondiabetic population.[55] Low level of hemoglobin levels in patients with diabetes adds in progression of kidney disease and cardiovascular mortality.[56] However, anemia in diabetes often goes unnoticed.[57] Treatment of rats with BR, HG, and BRHG mixture improved blood hemoglobin levels although it was unchanged in untreated SW rats. Significant changes in white blood cells were also noticed in rats receiving BR, HG, and BRHG mixture treatment.

These observations reveal that consumption of BR, HG, and BRHG mixture not only helps correct sucrose-induced disturbance in glucose and lipid metabolism but also corrects other biochemical and hematological abnormalities accrued due to sucrose toxicity.

Polyphenol-rich nutraceuticals and supplementary treatments have become therapeutics of interest for diabetics, because they have capacities of amending disturbed carbohydrate and lipid metabolism and improve insulin sensitivity. Polyphenols are also worked out in improving adipose tissue metabolism and play an important role in the alleviation of oxidative stress and stress-sensitive signaling pathways and inflammatory processes.[58] Our analysis shows that BR and HG powders are rich source of polyphenols and flavonoids and also possess capacities to scavenge various types of free radicals. Furthermore, the ability of these dietary grains in inhibiting formation of different types of advanced glycation end-products (AGEs) is of particular interest, because the formation of AGEs in hyperglycemia is responsible for the development of number of diabetic complications.[59,60]

Management of postprandial dysmetabolism and resultant oxidative stress requires particular attention in type 2 diabetes. For postprandial spikes in blood glucose and lipids instigate marked changes in redox status and are responsible for aggravating inflammatory stimuli.[19] Furthermore, oxidative stress in hyperglycemia has been recognized as a therapeutic target, for its alleviation plays important role in extenuating development of diabetic complications.[20,61] The imbalance between generation and scavenging of oxidants induces oxidative stress promoting disease and cell death.[29] Although the susceptibility of different cell types to oxidative stress-induced damage varies depending upon the concentration and

form of inducer, even the low concentration of H_2O_2 (10 μM) has been shown to induce oxidative stress in adult stem cells which was attenuated by addition of antioxidant Vitamin C.[29] Our study finds that the viability of HEK-293 and CHO cells was significantly compromised when they were incubated with 10 μM concentration of H_2O_2 for 48 h. It was observed that BR extracts could not significantly attenuate adverse effect of H_2O_2 treatment in HEK-293 cells; it was significantly attenuated by HG extract treatment at concentrations of 0.1 and 1.0 μg. Contrarily, however, higher concentration of HG extract (10 μg) failed to do so. On the other hand, different concentrations of BR and HG extracts were able to ameliorate significantly H_2O_2-induced effect on CHO cell viability. Antioxidants display paradoxical role in the context of oxidative stress;[62] therefore, care is warranted while delineating antioxidative stress potential of antioxidant concentrates and advocating therapeutic benefits of antioxidants.[63]

Besides macronutrients such as carbohydrate, protein, and fat, BR and HG powders are rich source of micronutrients such as vital vitamins, minerals, amino acids, fatty acids, and phytochemicals helpful in fighting disturbances caused due to dysglycemia and dyslipidemia. Vitamins and minerals play an important role in governing glucose metabolism and insulin signaling pathways and their absence in regular diet may contribute to the development of diabetes in obese population.[64] Dietary micronutrients and minerals are responsible for maintaining homeostasis in carbohydrate metabolism, insulin secretion, and its action, which have been found reduced in diabetic patients.[65,66] Unfortunately, however, their importance as therapeutics in diabetes often gets unnoticed.[66] Dietary supplementation of BR and HG powders to diabetes patients may meet these deficiencies.

Amino acid deficiencies have been observed in impaired glucose tolerant and type 2 diabetic individuals.[67] Supplementation of select amino acid has been reported to improve glucose tolerance in animal studies.[68] Ready-to-eat roasted fast-food made up of BR and HG contains in varying concentration up to 17 amino acids. Consumption of these food grains can suffice amino acids deficiencies responsible for maintaining proper homeostatic phenomenon governing glucose and lipid metabolism and insulin secretion and action. The observed effect of improvement in glucose tolerance in our study might be the collective effect of supply of micronutrients responsible for maintaining euglycemic and eulipidemic state in rats.

The connection between inflammation and obesity-linked insulin resistance, hyperglycemia, and hyperlipidemia refers diabetes as an inflammatory disorder.[69] Consumption of saturated fatty acids aggravates chronic inflammation and worsens insulin sensitivity,[70] whereas consumption of unsaturated fatty acids ameliorate hyperglycemia, improve insulin sensitivity,[71] and act as anti-inflammatory agents.[72-74] The presence of number of such unsaturated fatty acids in BR and HG powder might also be held responsible in reorganizing sucrose-induced disturbances in rats.

Very less is known about the duration of sucrose feeding, its influence on metabolic profiles, and development of full-fledged hyperglycemia, hyperlipidemia, or clear appearance of type 2 diabetes symptoms. However, short-term animal studies have proved that sucrose feeding induces dysglycemia, dyslipidemia, insulin resistance, impaired glucose tolerance, and weight gain marking symptoms of prediabetes.[16-18,75] Development of these abnormalities also depends on one's threshold capacity, responsiveness, and genetic susceptibility.[2] However, it is clear from our study that after 2 months, sucrose feeding and thereafter its withdrawal for a month induced symptoms of impaired glucose tolerance and disturbances in other biochemical and hematological parameters. Development of such symptoms was found resolved with treatment of rats with BR, HG, and BRHG mixture.

It is important to mention here that barley is a cereal grain and contains more carbohydrate, and horse gram is legume seed that contains more protein. The ratio of 3:2 of barley and horse gram, respectively, to prepare mixture of the two in our study was purely based on prevailing traditional practice. After roasting, these food grains are simply floured and become ready for consumption. Therefore, these fast-foods are scantly processed. Minimally processed foods produce low glycemic excursion and possess more satiety index.[9] With these criteria, BR and HG can become modern ready-to-consume food item providing less glycemic excursion with more satiety value and full of macro- and micro-nutrients.

CONCLUSION

This research provides scientific support to the ancient Indian medical prescription that consumption of BR can prevent development of diabetes and HG may be included in diabetic's food menu. Accordingly, they can be considered as ready-to-eat health food as food-bearing potentials of reversing disturbed metabolic functions in a positive direction are recognized as health food.[10] Apart from possessing macronutrients such as carbohydrates and proteins, these food grains also contain adequate amount of micronutrient vitamins, minerals, essential amino acids, fatty acids, and phytochemicals bearing multiple therapeutic properties. Therefore, these ready-to-eat fast-foods can also meet micronutrient deficiencies encountered during diabetes. To the best of our knowledge, this is the first research scientifically testifying beneficial role of BR and HG in dysglycemic and dyslipidemic conditions. Clinical validation of these scientific revelations is therefore warranted to prove their therapeutic health benefits.

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Conflicts of interest

There are no conflicts of interest.

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