

Dosakaya Juice Assuages Development of Sucrose Induced Impaired Glucose Tolerance and Imbalance in Antioxidant Defense

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ABSTRACT

Objective: The objective was to explore the effect of Dosakaya (DK) (*Cucumis melo* var. chito) juice on sucrose induced dysglycemia and disturbances in antioxidant defense in rats.

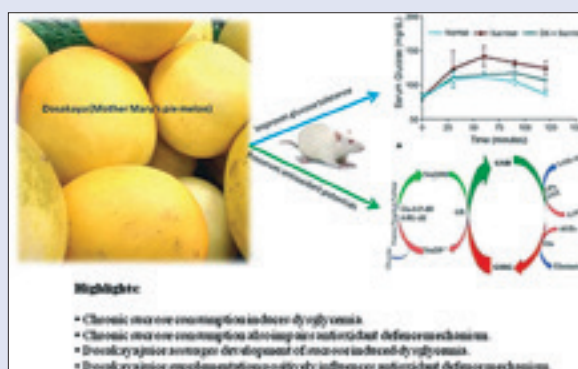
Materials and Methods: Rats were preconditioned with DK juice before administration of sucrose beverage continuously for 1-month. Blood glucose tolerance test and glutathione (GSH) homeostasis pathways in kidney were analyzed in different group of animals at the end of the study.

Results: DK juice diffused ($P < 0.001$) hypertriglyceridemia inducing effect of sucrose and arrested sucrose induced weight gain. It improved glucose tolerance ability by significantly reducing ($P < 0.05$) first-hour glycemic excursion and decreasing 2 h glycemic load ($P < 0.05$) following oral glucose tolerance test in sucrose fed animals. Furthermore, disturbances in antioxidant defense mechanisms in terms of GSH homeostasis in kidney were restored due to juice feeding. DK juice administration checked reduction in GSH-S-transferase and glyoxalase-I activity, thus, significantly mitigated lipid peroxidation ($P < 0.05$), and formation of advanced glycation end-products ($P < 0.001$) in kidney and serum ($P < 0.01$). Quantitative analysis of juice found it a rich source of protein and polyphenols. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis revealed the presence of multiple protein bands in whole fruit juice. Therefore, SDS-PAGE protein fingerprint of DK juice may serve as a quality control tool for standardization of juice. **Conclusion:** The whole fruit juice of DK may become cost-effective, affordable health beverage in extenuating ill-health effects of sugar consumption. This is the first report identifying DK juice in preventing development dysglycemia, dyslipidemia, and oxidative stress induced due to chronic sucrose feeding in rats.

Key words: Antioxidant activity, Dosakaya juice, glycemic excursion, impaired glucose tolerance, oxidative stress, protein-fingerprint, sucrose feeding, type 2 diabetes

SUMMARY

- Chronic sucrose consumption induced development of dysglycemia and also impaired antioxidant defense mechanism in rats. The oral administration of Dosakaya juice prior to sucrose feeding however, mitigated the development of dysglycemia and impairment in antioxidant defense in rats.



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INTRODUCTION

Despite the increase in understanding disease pathobiology and advances made in the field of medical sciences, modern world is still being engulfed by diseases of metabolic overload such as type 2 diabetes mellitus (T2DM), obesity, hypertension, cardiovascular disorders, and kidney diseases.^[1] Although, the consumption of high-caloric processed food items and decrease in physical activities are recognized as important factors driving such epidemics, the paradigm shift in research on food, nutrition, and health finds that increased consumption of sucrose (sugar) loaded food products and sugar-sweetened beverages in modern world further inflates the risk of diabetes, metabolic syndrome, and cardiovascular disorders.^[2] It has been reported recently that sugar consumption independently aggravates the development of chronic diseases linked to metabolic perturbations involving dysglycemia, dyslipidemia, and insulin resistance.^[3] It has also been observed that per capita sugar consumption is positively and independently associated with an increasing prevalence rates of T2DM worldwide and Asian regions in particular.^[4] Therefore, International Scientific Advisory

Committees on Nutrition, recommend limiting sugar consumption to 5% of daily calories to curb and or delay the development of diseases linked with metabolic overload.^[5]

On the other hand, researches dealing with food and food eating habits are finding that eating vegetables before carbohydrate-rich meals significantly reduces postprandial glycemic load, burden on pancreatic β cells to secrete insulin, improves postprandial glycemic excursions, and

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is helpful in long-term glycemic control in T2DM patients.^[6,7] Disclosing the health benefits of vegetable consumption, researchers find that they are rich natural sources of biological antioxidants and possess capabilities of maintaining glucose homeostasis by diverse mechanisms.^[8]

Dosakaya (DK) (*Cucumis melo* var. chito, Family: Cucurbitaceae) is cultivated prominently in the Southern parts of India. The fruit of DK, also known as Mother Mary's Pie Melon, is used by common people to prepare culinary items ranging from curries to pickles.^[9] It is a rich source of protein, polyphenols, and flavonoids, display multiple antioxidant activities, moderate starch-induced postprandial glycemic load in rats,^[10] inhibit protein-tyrosine phosphatase 1 β enzyme responsible for the development of insulin resistance,^[11] and influence polyol pathway by multiple mechanisms that may help prevent the development of diabetic complications.^[12] However, studies on chronic feeding of DK in dysglycemic animal models are not yet performed. In the present research, we report the effect of DK juice on the development of sugar induced dysglycemia and influence on antioxidant defense mechanisms in rats. Simultaneously, we also present sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) based protein fingerprint of whole DK fruit juice as a tool for standardization of the vegetable's juice along with quantitative analysis of total polyphenol, protein contents.

MATERIALS AND METHODS

Chemicals

1-chloro-2,4-dinitrobenzene (CDNB), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), nicotinamide adenosine dinucleotide phosphate (NADPH), acetic acid, oxidized glutathione (GSSG) and reduced glutathione (GSH), methylglyoxal and Folin-Ciocalteu phenol, and Bradford's reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo. USA). Other reagents of analytical grade were purchased from Indian manufacturers.

Sugar solution and Dosakaya juice preparation

The sugar solution was prepared daily in distilled water and orally administered to rats at a dose of 1.5 g/kg body weight.^[13] DK fruits were purchased from the local vegetable market. Fruits of Cucurbitaceae family sometimes taste bitter; therefore, pieces of DK fruit were self-tested and discarded if bitter. The raw DK fruit is delicious in nature and does not taste sweet. A paste of chopped pieces of DK fruit including seeds was made in food grade grinder and squeezed to get raw juice on a clean sterilized muslin cloth. Juice was orally administered to rats a fresh at 7.5 mL/kg body weight dose^[10] daily 30 min before sucrose feeding. For phytochemical analysis and SDS-PAGE protein-fingerprint, a paste of seeds was also prepared and after centrifugation, the supernatant was used for analysis.^[10]

Animals experiment

Adult male Wistar albino rats (180–220 g body weight) were purchased from Gentox Bio Services, Hyderabad, India. Rats were quarantined and acclimatized in institute's animal house (temperature $24 \pm 2^\circ\text{C}$, relative humidity 40–60%, and 12 h light/dark cycle). Normal rat chow pellets (Nutrimix Sp-1620, Nutrivet Life Sciences, Pune, India) and drinking water were provided *ad libitum*. Animal experiments were conducted following the ethical norms and CPCSEA guidelines. The experimental protocol was approved by Institutional Animal Ethical Committee (CPCSEA Reg. No. 97/1999 Government of India). Experiments with live animals comply relevant laws, and institutional guidelines and animals were handled humanly.

Rats were kept on overnight fasting and following day (forenoon) blood was collected from retro-orbital plexus in EDTA containing tubes. Plasma glucose levels ("0" h) were measured by glucose-oxidase

test (Siemens Healthcare Diagnostics Kit, Newark, USA) on auto-blood analyzer (Dimension Xpand plus Clinical Chemistry System, Siemens, Germany).

Rats were grouped (six rats in each group) based on their blood glucose levels as follows:

- Sugar control: Single dose 1.5 g/kg body weight sucrose solution per oral daily for a period of 1-month at forenoon time
- DK juice + sugar: Single dose (7.5 mL/kg body weight) freshly prepared DK juice followed by single dose (1.5 g/kg body weight) sucrose solution per oral was administered for a month. The gap between juice and sucrose feeding was 30 min
- Normal control: Rats were administered normal saline and treated sham.

Rats were kept in polypropylene rat cages with sterilized corn bedding. Food and water were provided *ad libitum* throughout the study.

Oral glucose tolerance test

At the end of 30 days experimental period, rats were kept for overnight fasting. The next morning "0" h blood samples were collected for estimation of hematological parameters, glucose, total cholesterol, and triglycerides. Hematological parameters were analyzed using Siemens Health care Diagnostic Kits (Newark, USA) on Advia 2120i Hematology analyzer (Siemens, Germany). Plasma glucose, total cholesterol, and triglycerides were analyzed using Siemens Healthcare Diagnostics Kits on the auto-blood analyzer. Glucose solution at the dose of 2 g/kg body weight was administered to all the rats. Blood was drawn at the intervals of 30 min for up to 120 min, and plasma glucose levels were estimated. Two-hour postprandial glycemic load^[10] and delta-glucose (ΔG) values^[14] were calculated accordingly.

Animals were euthanized under CO_2 anesthesia after completion of the experiment. Relevant organs were surgically removed, cleared of adventitious tissues, washed, and stored in -80°C Freezer (NUAIRE-2100, Ultraflow freezer, Plymouth, MN, USA) for further analysis.

Enzymatic and biochemical analysis

Tissue preparation

Kidney tissues (50 mg) were chopped and homogenized (Heidolph Schwabach, Germany) in 500 μL of PBS (0.1 M, pH 7.2) with the addition of cocktail protease inhibitor. Centrifugation (Beckman Coulter Avanti J-301 centrifuge, California, USA) was done at 15,000 rpm for 30 min at 4°C . The supernatant was stored at -80°C for analysis.

Estimation of reduced glutathione

GSH was measured in the kidney following Ellman method.^[15] A volume of 100 μL supernatant was reconstituted in 2 mL of phosphate buffer (0.3 M, pH 8.0), 500 μL DTNB (2 mg DTNB + 1 g sodium citrate dissolved in 100 mL water), mixed thoroughly and incubated for 10 min. The intensity of yellow color was measured spectrophotometrically (Perkin Elmer^{precisely} Labda 25, UV-Vis Spectrophotometer, Waltham, MA, USA) at 412 nm. Results were expressed as mM.

Glutathione reductase

The method for estimation of glutathione reductase (GR) was adapted from Carlberg and Mannervick.^[16] The kinetics of reaction mixture containing 150 μL of GSSG (2 mM), 50 μL of assay buffer (0.1 M KH_2PO_4 + 1 M EDTA, pH 7.0), 30 μL sample, and 20 μL NADPH (2 mM) in a 96-well plate was read at 340 nm for 3 min at 30 s interval spectrophotometrically (BioTek^{synergy4} Multimode Microplate Reader, BioTek Instruments Inc., Winooski, VT, USA). NADPH utilization was considered as a measure of enzyme activity. The results were expressed as $\Delta\text{OD}/\text{min}$.

Glutathione-S-transferase (GST)^[17] method was applied for measurement of GST activity. Twenty microliter supernatant was reconstituted in reaction mixture containing 150 μ L of phosphate buffer (0.1 M, pH 6.5, 20 μ L of GSH (1 mM) and 10 μ L of CDNB (1 mM) in a 96-well plate. Reaction kinetics was recorded every 1 min for 5 min at 340 nm spectrophotometrically. Results were expressed as Δ OD/min.

Glutathione peroxidase analysis

Glutathione peroxidase (GPx) activity in kidney supernatant was measured following the method of Rotruck *et al.*^[18] with suitable modifications. Briefly, 100 μ L each of GSH (4 mM), sodium azide (10 mM), EDTA (0.8 mM), and hydrogen peroxide (30 mM) were incubated for 10 min at 37°C in the presence of 20 μ L of respective kidney supernatant. The mixture was centrifuged at 3000 rpm for 10 min after addition of 500 μ L trichloroacetic acid (10%). Hundred microliters DTNB (0.04%) was added to the supernatant. Absorbance was recorded at 420 nm and expressed as enzyme activity.

Assay of glyoxalase-I activity

Glyoxalase-I (Glo-I) activity was assayed as described by Thornalley^[19] with suitable modifications. In brief, hemithioacetal was prepared by mixing 500 μ L of 2 mM GSH in sodium phosphate buffer (100 mM, pH 6.6) with 500 μ L methylglyoxal (2 mM) and incubated for 10 min at 37°C. The reaction was started by addition of 20 μ L kidney supernatant as a source of enzyme. The conversion of hemithioacetal into S-D-lactoylglutathione due to Glo-I was measured spectrophotometrically (240 nm). Results were expressed as an increase in absorbance as a function of enzyme activity.

Analysis of thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances as a measure of lipid peroxidation products were measured as described by Wills.^[20] In 5 mL glass test tube, 100 μ L supernatant, 200 μ L sodium dodecyl sulfate (8.1%), 1.5 mL acetic acid (30%, pH 3.5), 1.5 mL TBA were mixed thoroughly and incubate in boiling water-bath for 60 min at 95°C and cooled. Two hundred microliter clear solution was transferred to a 96-well plate, and absorbance (535 nm) was recorded spectrophotometrically. Results were expressed as nM/g tissue.

Advance glycation end-products analysis

150 mg chopped kidney tissues were soaked in chloroform-methanol (2:1) mixture and incubated (4°C) overnight in shaker incubator (Innova 4230 refrigerated incubator shaker, New Brunswick Scientific, Champaign, IL, USA). Filtered (0.2-micron filter) pellets were washed thrice with methanol and water. Tissues were homogenized in 1 mL NaOH (0.1 N) and centrifuged at 8000 rpm for 15 min at 4°C. Fluorescence (excitation 370 nm and emission 440 nm) was recorded.^[21] Results were expressed in fluorescent units.

Analysis of advanced glycation end-products in serum

Serum was deproteinized by mixing with an equal volume of acetonitrile and incubated for 2 min (–20°C) and centrifuged for 10 min at 9500 rpm (4°C). Fluorescent advanced glycation end-products (AGEs) in the supernatant were assayed (excitation 370 nm and emission 440 nm) as reported earlier.^[13] Results were expressed in fluorescent units.

Estimation of total polyphenols

A volume of 25 μ L supernatants from whole fruit juice or the seeds was diluted with 2.5 mL distilled deionized water. Equal volumes of Folin–Ciocalteu reagent and Na₂CO₃ were mixed to this and incubated for 60 min. Absorbance (765 nm) was recorded, and results were expressed as μ g gallic acid equivalent/mL as described earlier.^[10]

Estimation of total protein

Protein content in juice, as well as seeds, was determined using Bradford's dye as described earlier.^[10] Briefly, 10 μ L samples were reacted with 240 μ L of $\times$ 1 Bradford reagent, and absorbance (595 nm) was recorded. Protein concentration was expressed BSA equivalent/mL.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis protein-fingerprinting of whole juice and seeds

Protein-fingerprint was prepared as described earlier^[10] with suitable modifications. Supernatant of whole fruit and seed of DK were mixed with ice-cold acetone (100% v/v) and trichloroacetic acid (100% w/v) in the ratio of 1:8:1 and kept at –20°C for 1 h for protein precipitation. Pellet was collected after centrifugation at 15,000 rpm at 4°C for 15 min. Pellet was suspended in 1 mL ice-cold acetone and centrifuged again at 15,000 rpm 4°C for 15 min. This step was repeated three times to remove the trichloroacetic acid. Pellet was dried to remove acetone. Dried protein pellets were dissolved in PBS. Protein concentration was measured using Bradford's reagent. Forty microgram protein was mixed with 7 μ L of nonreducing ($\times$ 4) lithium dodecyl sulfate sample buffer (Thermo Scientific, USA) and heated at 95°C for 5 min in dry bath and cooled at room temperature. Twenty micro gram protein were loaded on 10% SDS-PAGE (Bio-Rad protein gel apparatus) along with 5 μ L molecular weight marker (Biomatik, USA). The gel was run in trisglycine buffer, at constant voltage (120 V). Coomassie brilliant blue (0.5%, 250 R) stained gel was de-stained several times with methanol: acetic acid: water (40:10:50) mixture and scanned with scanner (HP LaserJet M1136 MFP, USA)

Statistical analysis

A one-way analysis of variance followed by Tukey's multiple comparison tests was applied to find differences in study groups. The criterion for statistical significance was $P < 0.05$. Statistical analyses were performed using GraphPad PRISM® version 5.01 (La Jolla, USA).

RESULTS AND DISCUSSION

Mortality due to feeding sugar alone or DK juice + sugar was not found during the study period. Rats in normal and DK juice + sucrose were active throughout the study, however, animals in sucrose fed group looked sluggish 15 days onward.

Hematological and biochemical observations in blood

DK juice, as well as sucrose feeding for a month, did not bring any significant changes in blood cells and related parameters [Table 1]. Although, an increase in body weight was found in sucrose fed animals, it was not statistically significant. Similarly, no significant changes in fasting plasma glucose and total cholesterol levels were noticed at the end of the study. However, the plasma triglyceride levels were significantly ($P < 0.01$) increased in rats receiving sucrose when compared with a normal group of rats. Fasting plasma triglyceride levels in rats receiving DK juice before sucrose feeding was found significantly ($P < 0.05$) lesser when compared with a normal group of animals and sucrose fed rats ($P < 0.001$). Results indicate that DK juice possess anti-hypertriglyceridemic activity by defusing hypertriglyceridemic effects of sucrose and also possess hypotriglyceridemic activity since it reduced triglyceride levels below normal values [Table 1].

Oral glucose tolerance test

The shape of plasma glucose concentration curve following oral glucose tolerance test (OGTT), foretells risk of T2DM development in a person

in future.^[22] Based on the shape of plasma glucose concentration curve following OGTT, it has been found that if plasma glucose concentration does not return to basal level, person may bear the characteristics of impaired glucose tolerance (IGT), insulin resistance, compromised insulin sensitivity, and hence, individual is considered as a case of prediabetes.^[22] Prediabetes state often progresses to overt diabetes within few years.^[23] Therefore, an early lifestyle modifications can reverse back prediabetes state to normal state and attenuate diabetes progression. Furthermore, it is advised to maintain postprandial glucose curve flat^[24] because postprandial hyperglycemia (PPHG) is one of the earliest detectable abnormalities in diabetes prone individuals.^[25]

Our research clearly demonstrates that continuous administration of sucrose for 1-month induced IGT development in rats [Figure 1a]. Interestingly, however, prior administration of DK juice substantially curtailed this effect and significantly ($P < 0.05$) reduced postprandial glycemic load following OGTT [Figure 1d].

Glucose fluctuations during the postprandial period are recognized to trigger oxidative stress.^[26] Slama *et al.*^[27] proposed that postprandial glycemic excursion plays an important role in total hyperglycemia. Therefore, the

measurement of Δ -postprandial glycemia (ΔG) is a more useful tool than the conventional examination of absolute rise in the postprandial blood glucose level. Furthermore, control of acute glucose surge is emerging as a new therapeutic tool toward minimizing hyperglycemia-induced oxidative stress and progression of diabetic complications.^[26]

The first-hour glucose surge ($\Delta G_{60-0 \text{ min}}$) following OGTT is presented in Figure 1b. Glycemic spikes during the first hour were significantly ($P < 0.05$) higher in sucrose fed animals than in a normal group. On the other hand, the first-hour glycemic surge was significantly ($P < 0.05$) lesser in rats receiving DK juice along with sucrose and was close to the normal group of rats. Contrarily however, in the later half ($\Delta G_{60-120 \text{ min}}$), the decrease in blood glucose level following OGTT in DK group was lesser than the reductions observed in rats receiving only sucrose [Figure 1c]. This reduction was prominent in the normal group of rats and blood glucose level reached close to basal values [Figure 1a]. Wide individual variations in blood glucose surge and deceleration were noticed in rats fed with sucrose and DK juice + sucrose following OGTT. These variations may arise due to their individual responsiveness and susceptibility to sucrose treatment, as well as DK juice.

Table 1: Values of hematological and biochemical parameters of rats at the end of the study

	BW (g)	RBC ($10^6 \text{ cell}/\mu\text{L}$)	MCV (fL)	HCT (%)	PLT ($10^3 \text{ cell}/\mu\text{L}$)	WBC ($10^3 \text{ cell}/\mu\text{L}$)	HGB (g/dl)	MCH (pg/dl)	MCHC (g/dl)	PG (mg/dl)	PTC (mg/dl)	PTG (mg/dl)
N	305 $\pm$ 9	7.01 $\pm$ 0.19	52.7 $\pm$ 1.6	36.92 $\pm$ 0.43	757.8 $\pm$ 42.4	14.58 $\pm$ 1.36	13.7 $\pm$ 0.1	19.58 $\pm$ 0.58	37.08 $\pm$ 0.22	84.3 $\pm$ 3.8	61.2 $\pm$ 2.1	66.7 $\pm$ 3.5
SC	330 $\pm$ 9	7.32 $\pm$ 0.2	50.14 $\pm$ 0.89	36.64 $\pm$ 0.56	723.6 $\pm$ 41	20.44 $\pm$ 1.75	13.6 $\pm$ 0.2	18.64 $\pm$ 0.34	37.22 $\pm$ 0.33	82.8 $\pm$ 5.9	63 $\pm$ 2.1	87.9 $\pm$ 3.8
DK+SC	309 $\pm$ 7	7.7 $\pm$ 0.3	49.5 $\pm$ 0.56	38.12 $\pm$ 1.29	622.8 $\pm$ 66.1	15.6 $\pm$ 2.6	14.4 $\pm$ 0.5	18.26 $\pm$ 0.26	36.9 $\pm$ 0.18	82 $\pm$ 4.0	58.6 $\pm$ 2.4	51.6 $\pm$ 4.3

Values represent mean $\pm$ SEM, $n=6$. ^a $P < 0.01$ compared with SC; ^b $P < 0.001$ compared with DK+SC; ^c $P < 0.05$ compared with N. N: Normal control; SC: Sucrose; DK+SC: Dosakaya+sucrose; BW: Body weight; RBC: Red blood cells; MCV: Mean corpuscular volume; HCT: Hematocrit; PLT: Platelet; WBC: White blood cells; HGB: Hemoglobin; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PG: Plasma glucose; PTC: Plasma total cholesterol; PTG: Plasma triglycerides; SEM: Standard error of mean

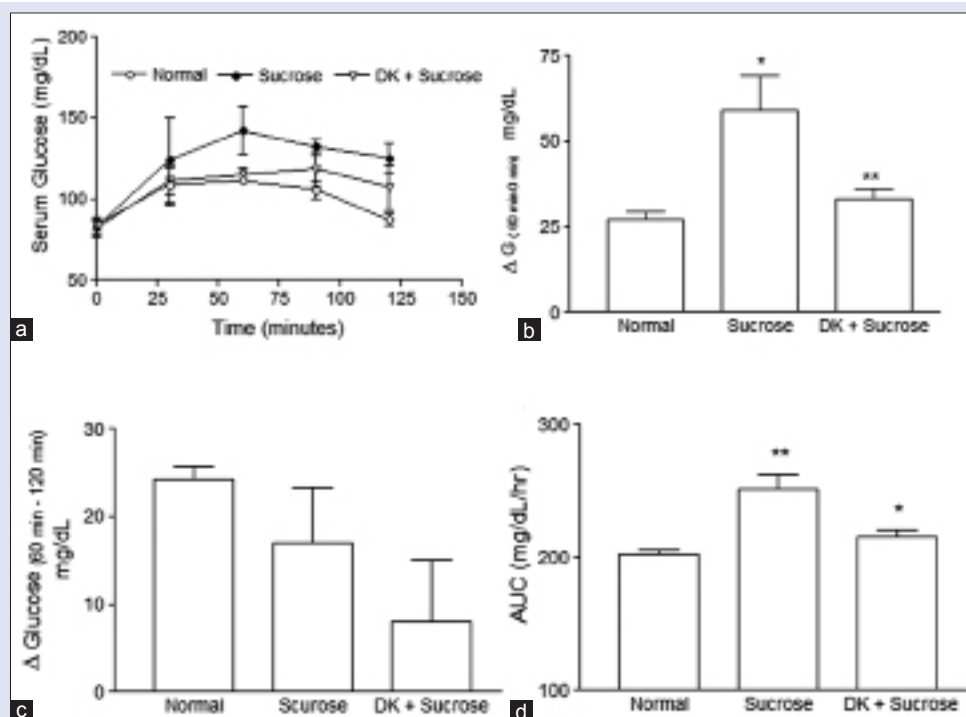


Figure 1: Oral glucose tolerance test in rats of different experimental groups at the end of 30 days experimental period. (a) The shape of plasma glucose concentration curve at different time points following oral glucose tolerance test. (d) Glycemic load (area under the curve, AUC) following oral glucose tolerance test. ^{**} $P < 0.01$ normal versus sucrose, ^{*} $P < 0.05$ sucrose versus Dosakaya + sucrose. (b) Glycemic spikes during the first hour ($\Delta G_{60-0 \text{ min}}$) following oral glucose tolerance test. ^{*} $P < 0.05$ normal versus sucrose, ^{**} $P < 0.05$ sucrose versus Dosakaya + sucrose. (c) Glycemic spikes between 60 and 120 min ($\Delta G_{60-120 \text{ min}}$) following oral glucose tolerance test. Values represent mean $\pm$ standard error of mean, $n = 5-6$

Antioxidant defense

PPHG is an important factor in increasing free radicals generation and oxidative stress even in nondiabetic individuals.^[28] Accelerated generation of free radicals and consequent oxidative stress is an important link between diabetes and development of cardiovascular diseases.^[29] Prolonged and high consumption of sucrose and fructose induces development of oxidative stress.^[13] Therefore, arresting development of oxidative stress due to hyperglycemia presents exciting therapeutic opportunity in mitigating onset of diabetic complications.

Furthermore, antioxidant defense in body is governed by complex mechanisms working in coordination with a number of pathways. For example, GSH works in coordination with other redox-cycles operating in the body to maintain and regulate cellular redox balance [Figure 2]. GSH performs multiple functions and displays antioxidant activities in various ways.^[30] Therefore, maintenance of GSH homeostasis is an important consideration for the balanced antioxidant defense.

A significant ($P < 0.05$) increase in GSH concentration was recorded in kidney tissues of rats receiving DK juice along with sucrose when compared with normal rats [Figure 3a]. GSH is regenerated from oxidized GSSG by the enzyme GR in coordination with NADPH cycle primarily originating from the pentose phosphate pathway [Figure 2]. Results presented in Figure 3b show that although the GR activity was augmented in sucrose fed rats, this increase was significantly ($P < 0.05$) higher than normal rats in animals receiving DK juice along with sucrose [Figure 3b]. The augmentation in GR and GSH levels in sucrose fed animals than normal group of rats may be linked to the energetic system related to pentose phosphate pathway, whereas increased glucose concentration due to sucrose feeding might be providing enough NADPH through the pentose phosphate pathway [Figure 2]. As, DK juice is considered as rich

source of antioxidants,^[10,12] significant increase in levels of antioxidants GR and GSH in DK juice + Sucrose group of animals appears advantage of DK juice feeding [Figure 3a and b]. The increase in GR levels due to DK juice in rats can be seen as an improvement in antioxidant defense because; GR protects cells from harmful effects of hydroperoxides.^[31]

Enzymes GST and GPx are responsible for the protection against various genotoxic agents and peroxide radicals in the cells.^[30] A significant decrease ($P < 0.05$) in GST level from normal levels in sucrose fed rats was observed [Figure 3c] in our study. However, preconditioning of rats by DK juice feeding prevented the reduction in GST level due to sucrose, in kidney tissues [Figure 3c]. We did not observe appreciable changes in GPx level in our study [Figure 3d].

GSH also acts as a cofactor in systems responsible for the elimination of AGEs in association with glyoxalases.^[32] Thornalley^[19] reported decreased activity of Glo-I in hyperglycemic conditions. The decrease in Glo-I activity, therefore, may compromise elimination of AGEs and promote their accumulation. A significant decrease ($P < 0.05$) in Glo-I activity after sucrose feeding was noticed in our study also [Figure 3e]. However, the level of Glo-I was maintained close to the normal in rats receiving DK juice before sucrose [Figure 3e]. These observations find that DK juice prevents imbalance in antioxidant homeostasis induced due to sucrose feeding and may help prevent damage to biomolecules and their accumulation.

Reduction in GST and Glo-I activities due to sucrose feeding might be held responsible for increased levels of lipid peroxides [Figure 3f] and AGEs in the kidney [Figure 3g]. For DK juice feeding retained activities of GST and GPx, so the values of lipid peroxides were recorded significantly ($P < 0.05$) lesser [Figure 3f]. However, it was still higher ($P < 0.001$) than normal animals.

Hyperglycemia and accompanied oxidative stress accelerate formation of AGEs. Increased generation and accumulation of AGEs are responsible for aggravating development of diabetic complications.^[29] Significant increase in AGEs were recorded in kidney (Figure 3g, $P < 0.001$), as well as in serum (Figure 3h, $P < 0.01$), of sucrose fed rats than the level recorded in normal group of rats [Figure 3g and h]. These observations find support from our earlier observations that sustained hyperglycemia induces oxidative stress and increases the formation of AGEs.^[13] The DK juice significantly prevented the formation of AGEs, both in kidney (Figure 3g, $P < 0.001$), as well as in serum (Figure 3h, $P < 0.01$).

Phytochemical analysis and sodium dodecyl sulfate polyacrylamide gel electrophoresis based protein-fingerprints

Polyphenols and proteins present in plant materials are reported to possess antioxidant activities.^[33,34] Furthermore, a high protein concentration in vegetables is held responsible for the anti-hyperglycemic activity.^[10] Our analysis finds that whole fruit juice, as well as seeds of DK, is rich in polyphenols and proteins. The polyphenol and protein concentration in seeds were recorded significantly higher ($P < 0.05$ and $P < 0.001$, respectively) than in whole fruit juice [Figure 4a].

One of the major challenges associated with natural products is lack of suitable standardization technique leading increase in adulteration malpractices. The unique banding pattern of protein electrophoregram of individual food material can provide biometric data for their identification and standardization of products.^[10,35] The SDS-PAGE protein-fingerprint [Figure 4b] of whole fruit, as well as seeds of DK, may serve this purpose.

Changes in lifestyle and dietary patterns from traditional to modern are being held responsible for the outburst of hyperglycemia and hyperlipidemia in modern society. Therefore, simple realignment of our

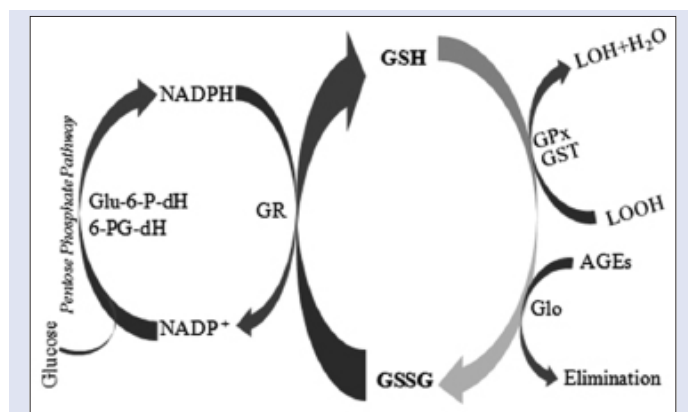


Figure 2: Scheme represents glutathione homeostasis. The reduced glutathione and oxidized glutathione forms of glutathione work in coordination with other redox-cycles (e.g. nicotinamide adenosine dinucleotide phosphate) to maintain and regulate cellular redox balance. Glutathione reductase reduces glutathione disulfide to sulfhydryl form (glutathione) by nicotinamide adenosine dinucleotide phosphate-dependent mechanism. Nicotinamide adenosine dinucleotide phosphate is primarily available via pentose phosphate pathway involved in glucose oxidation. Glutathione acts directly as antioxidant and also as cofactor for various enzymes such as glutathione-S-transferase responsible for protection against various genotoxic and carcinogenic compounds, glutathione peroxidase accountable for reduction of various peroxides (LOOH, H_2O_2) and glyoxalases involved in elimination of advanced glycation end-products. An imbalance in the activities of antioxidant enzymes such as glutathione reductase, glutathione peroxidase, glutathione-S-transferase, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase may affect cellular defense system. NADP⁺: Nicotinamide adenine dinucleotide, LOOH: Lipid peroxides

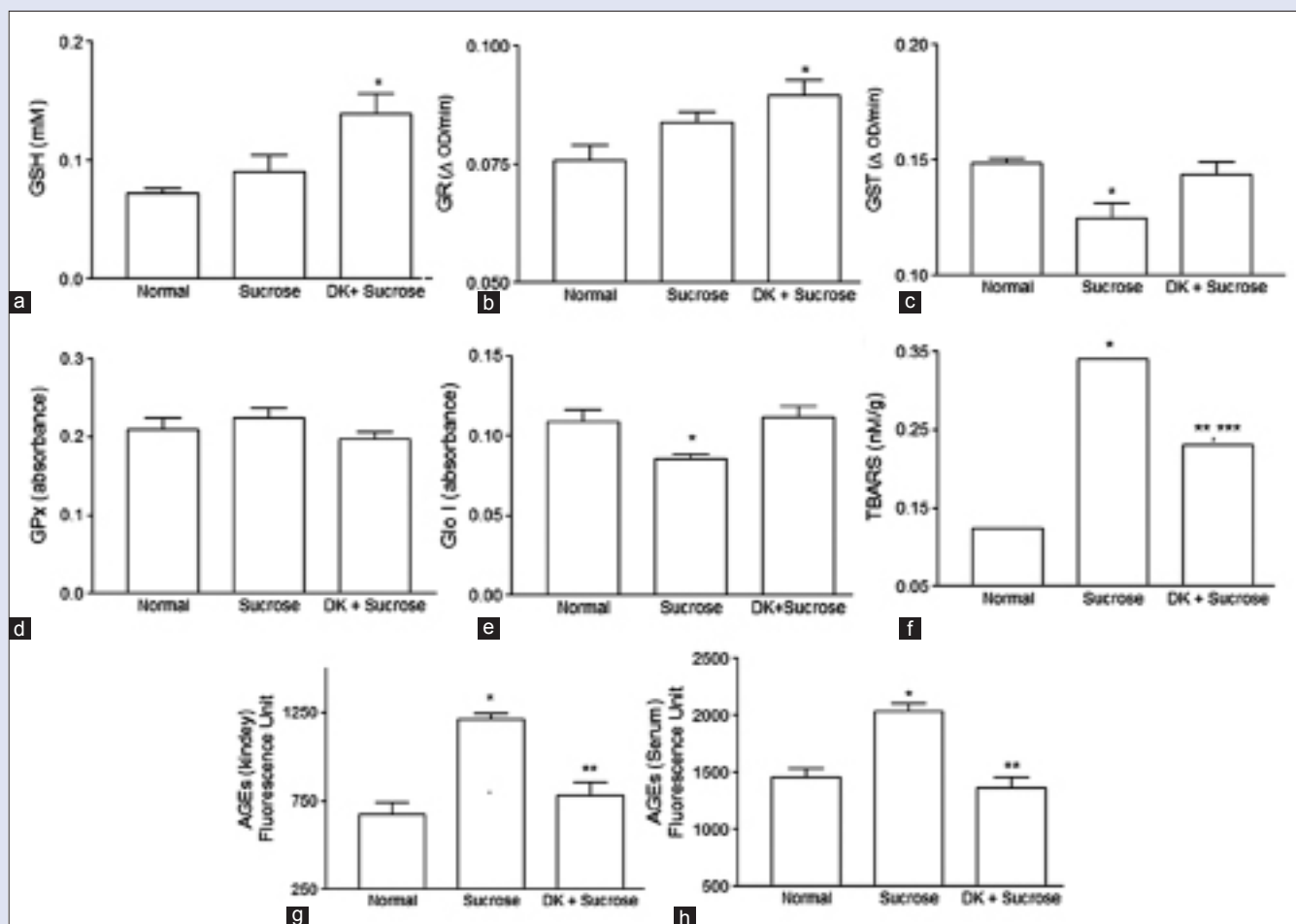


Figure 3: The effect of Dosakaya juice on various antioxidant parameters related to glutathione homeostasis in kidney under the influence of sucrose feeding. (a) Concentration of reduced glutathione in various experimental groups, * $P < 0.05$ normal versus Dosakaya + sucrose. (b) The level of glutathione reductase, * $P < 0.05$ normal versus Dosakaya + sucrose. (c) The level of Glutathione-S-transferase, * $P < 0.05$ normal versus sucrose. (d) The activity of glutathione peroxidase. (e) The activity of glyoxalase-I (Glo I), * $P < 0.05$ normal versus sucrose. (f) Thiobarbituric acid reactive substances (TBARS) as measure of lipid peroxides in kidney, * $P < 0.001$ normal versus sucrose, ** $P < 0.05$ sucrose versus Dosakaya + sucrose, *** $P < 0.05$ normal versus Dosakaya + sucrose. (g) Advanced glycation end-products (AGEs) in kidney, * $P < 0.001$ normal versus sucrose, ** $P < 0.001$ sucrose versus Dosakaya + sucrose. (h) Advanced glycation end-products (AGEs) in serum, * $P < 0.01$ normal versus sucrose, ** $P < 0.01$ sucrose versus Dosakaya + sucrose. Data represent mean $\pm$ standard error of the mean of 5–6 animals. Readings were taken in triplicate for each animal

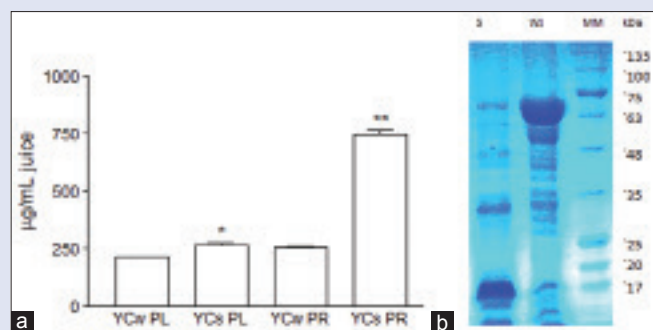


Figure 4: Phytochemical analysis in whole juice and seeds of Dosakaya. (a) Total polyphenol (PL) and protein content (PR) in whole juice (wj) and seeds (s), * $P < 0.05$ wj versus s and ** $P < 0.001$ wj versus s values represent mean $\pm$ standard error of mean of at least three experiment. (b) Sodium dodecyl sulfate polyacrylamide gel electrophoresis protein fingerprint of wj and s of Dosakaya

dietary habits with natural food materials may provide holistic health benefits without side effects. In this regard, preventive effect offered by DK juice against the development of IGT, and imbalance in antioxidant defense induced due to sugar consumption may become cost effective alternative natural substitute at the place of sugar-sweetened beverages. Further research is required, however, to translate health promoting effect of DK juice in clinical conditions. To the best of our knowledge, this is the first scientific evaluation and health promoting effect of DK juice, which may be helpful in preventing development of dysglycemia and oxidative stress induced by consumption of sugar-sweetened beverages.

CONCLUSIONS

The whole fruit juice of DK may become cost effective, affordable health beverage in extenuating ill-health effects of sugar consumption. This is the first report identifying DK juice in preventing development of dysglycemia, dyslipidemia, and oxidative stress induced due to chronic sucrose feeding in rats.

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Conflicts of interest

There are no conflicts of interest.

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