Preventive and Therapeutic Efficacies of *Benincasa hispida* and *Sechium edule* Fruit’s Juice on Sweet-beverages Induced Impaired Glucose Tolerance and Oxidative Stress

1Ashok Kumar Tiwari, 1Iragavarapu Anusha, 1Maddineni Sumangali, 1Domati Anand Kumar, 1Kuncha Madhusudana and 1,2Sachin Bharat Agawane
1Medicinal Chemistry and Pharmacology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad-500007, India
2Biochemical Sciences Division, CSIR-National Chemical Laboratory, Pune-411 008, India

**ABSTRACT**

**Aim:** Increased consumption of sugar-sweetened beverages have been identified as one of the major sources responsible for increasing global epidemic of hyperglycemia, hyperglycemia induced oxidative stress and consequently, development of number of diabetic complications. This research explored preventive and therapeutic potentials of fruit juice of *Benincasa hispida* (BH) and *Sechium edule* (SE) in fructose and sucrose-sweetened beverages induced Impaired Glucose Tolerance (IGT), oxidative stress and IGT induced other metabolic disturbances in rats. **Materials and Methods:** Rats were orally administered fructose and sucrose sweetened-beverage prepared in water and respective fruit’s juice for one month. A group of rats were maintained for three months on daily single dose of sucrose. Three months sucrose-fed rats were later treated with fruit’s juice for fifteen days. Degree of IGT development was determined following Oral Glucose Tolerance Test (OGTT). Shape of plasma glucose concentration curve following OGTT, glycemic load, plasma Total Antioxidant Potential (TAOP), oxidative stress status, platelet aggregation activity and, Advanced Glycation End-products (AGEs) level in blood of rats were examined. **Results:** Fructose was more potent in inducing IGT than sucrose. Sucrose was more potent in decreasing plasma TAOP and inducing oxidative stress than fructose. BH juice prevented development of IGT induced by fructose and also significantly (p<0.05) reduced two hour glycemic load following OGTT. Three months continuous single dose administration of sucrose in rats induced development of IGT, increased two hour glycemic load (p<0.05) following OGTT, decreased TAOP (p<0.05), increased oxidative stress (more than twice) when compared with normal rats. Treatment of theses rats with fructose or Sucrose SE accelerated normalization of oral glucose tolerance ability and decreased oxidative stress, platelet aggregation and accumulation of AGEs in sucrose induced IGT and oxidatively stressed rat. **Conclusion:** Our observations reveal that consumption of BH and SE juice may help reduce development of hyperglycemia and hyperglycemia induced development of complications by virtue of their antihyperglycemic and antioxidative stress potentials.

**Key words:** *Benincasa hispida*, *Sechium edule*, hyperglycemia, impaired glucose tolerance, oxidative stress, platelet aggregation, advanced glycation end products, sugar-sweetened beverages


**INTRODUCTION**

Modern epidemic of type 2 diabetes mellitus (T2DM) is considered to be the consequence of changes in human behavior such as consumption of high amount of calorie-dense, energy-rich nutrition followed by adoption of sedentary life style. T2DM is emerging at epidemic rate in many parts of the world including developed nations like United States of America and developing countries like China and India. Increasing evidences suggest that rise in consumption of sugar-sweetened beverages and food items enriched with high ratio of fructose is one of the important contributing factors exaggerating development of T2DM. Although, the disaccharide sucrose is predominant sugar in most of the modern foods, the monosaccharide sweetener fructose is very common in a wide range of food products in some countries like USA. Increased amount of fructose in diet is identified as an important causative factor responsible for increasing insulin resistance.
decreasing insulin sensitivity and consequently, development of Impaired Glucose Tolerance (IGT). High-fructose diet is reported to induce oxidative stress by decreasing antioxidant defense and increasing free radicals generation. Furthermore, postprandial hyperglycemic excursions (PPHGE) and hyperglycemic spikes even in non-diabetic individuals also increase oxidative stress. Postprandial hyperglycemic spikes instigate redox imbalance in short term and lead to the development of complex chronic diseases in long-term exposure.

Fruits and vegetables are being identified as potential readily available and cost-effective resources for development of new generation therapeutics for lifestyle related disorders. Therefore, identification and study of fruits and vegetables that possess potentials to mitigate diet-induced IGT development, diminish oxidative stress level and correct IGT and oxidative stress induced biochemical and physiological imbalances may hold promise in reducing risks responsible for development of T2DM and diabetic complications.

Recommendations for inclusion of coarse cereals, fruits and vegetables in human diet have increased recently because such food items contain variety of potential biological antioxidants. However, health benefits of polyphenols-rich dietary supplements, dietary antioxidant supplements and antioxidant compounds from natural resources have been questioned recently for their prooxidant behaviors. Observations that consumption of polyphenolic-rich fruits increases antioxidant capacity of blood and counterbalances prooxidant effects induced by high carbohydrate and fat meals has been challenged by reports indicating that augmentation of blood antioxidant potential may be due to fructose-induced increase in serum uric acid level rather than polyphenols present in fruits-juice. Although, uric acid possesses antioxidant properties, it is also a risk factor for several diseases. In addition, some antioxidant-rich fruits augment hyperlipidemia and antioxidant-rich fraction of some cereals and dietary formulations display proglycemic activity. Vegetables are reported to be rich source of biological antioxidants and serve cost effective culinary item. However, high polyphenolic content in vegetable’s juice was observed recently to augment starch induced PPHGE.

These reports therefore, warrant indiscriminate use of polyphenols-rich dietary antioxidant supplements and demand search of dietary materials that mitigates development of diet-induced glycemic burden and also reduces level of oxidative stress. Single dose oral administration of antioxidants rich juice of Benincasa hispida (BH) and Seshium edule (SE) was identified to mitigate starch induced PPHGE in rats. Although, BH is extensively used in traditional oriental medicine for treatment of gastrointestinal problems, respiratory, heart and urinary diseases and SE advocated for treatment of arteriosclerosis, hypertension and inflammation, effect of the juice of these fruits is not explored in sugar-sweetened beverages induced hyperglycemic conditions. In the present study, we report evaluation of preventive and therapeutic efficacy of BH and SE fruits juice on fructose and sucrose induced development of IGT and oxidative stress in rats.

MATERIALS AND METHODS

Chemicals: 2', 2'-Aminobis-3-ethyl benzthiazoline-6-sulfphonic acid diammonium salt (ABTS), Gallic acid, Rutin, Bradford and Folin-Ciocalteu reagents were purchased from Sigma-Aldrich chemicals (St Louis, MO, USA) and Platelets aggregation kits from Chronolog Corporation, USA. Kits for analysis of plasma glucose were obtained from Siemens Healthcare diagnostics Ltd, Frimley, Camberley, UK. Other chemicals like fructose, sucrose and potassium persulfate etc were purchased from Indian manufacturers.

Preparation of juice: Fruits of Benincasa hispida Thurb. Cogn. (Family Cucurbitaceae) and Seshium edule L. (Family Cucurbitaceae), were procured daily from local vegetable markets (Hyderabad, India). Fruits were washed thoroughly with water and cut into pieces. Juice was obtained by grinding in food-grade grinder and squeezed to maximum amount with clean muslin cloth.

Analysis of chemical components in juice: Chemical components like total polyphenols, flavonoids, anthocyanins and protein concentrations in fresh juice of these vegetables were analyzed as follows:

- **Total polyphenolic content:** Total polyphenolic content in juice was measured using Folin-Ciocalteu reagent. Briefly, fresh (25 µL) juice was diluted with 2.5 mL distilled de-ionized water followed by addition of 250 µL of Folin-Ciocalteu reagent (1 M) and 250 µL of Na,Co, (20% w/v). Mixture was incubated at room temperature (60 min). Absorbance (765 nm) was recorded spectrophotometrically on microplate reader (SpectraMax plus Molecular Devices, Sunnyvale, CA). Total polyphenolic content was expressed as milligrams of Gallic Acid Equivalent (GAE)/µL juice.
- **Total anthocyanins:** Anthocyanins in juice were determined as described by Giusti et al. To 25 mM potassium chloride solution (pH 1.0) and
0.4 M sodium acetate buffer (pH 4.5), equal volumes of fresh juice were added. Absorbance was measured at 510 and 700 nm. Data was expressed using molecular extinction coefficient, molecular weight of anthocyanins and an absorbance of \( A = \frac{(A_{400} - A_{600}) \text{pH 1.0} - (A_{400} - A_{600}) \text{pH 4.5}}{100} \) as milligrams of anthocyanins per 100 mL juice

- **Total flavonoids**: Total flavonoids content in samples was measured by mixing equal volume of vegetable’s juice with 2% \( \text{AlCl}_3 \cdot 6\text{H}_2\text{O} \) in a 96 well micro plate\(^1\). Absorbance was recorded spectrophotometrically at 430 nm. Results were expressed as milligrams of rutin Equivalent (RE)/mL juice

- **Total protein content**: Protein content in juices was determined using Bradford’s reagent\(^2\). Briefly, 10 \( \mu \)L juice was mixed with 240 \( \mu \)L of Bradford reagent and absorbance was read at 595 nm spectrophotometrically. Results were expressed as milligrams BSA Equivalent/mL juice

**Animal experiments**: Animal experiments were performed on adult male Wistar rats (170-210 g b.wt.). Institutional Animal Ethical Committee (CPCSEA Reg. No.97/1999, Government of India) approval for experimental protocol was obtained. Experiments with live animals were performed in compliance with relevant laws and institutional guidelines. Rats were housed three per cage in polystyrene cages (with sterilized rice-husk bedding changed daily) in temperature-controlled room with 12 h light: dark cycle in institute’s animal house. Normal rat chow (Nutrimix Sp-1620, Nutrivet Life Sciences Pune, India) and water was provided ad libitum throughout experimental period.

On day one of experiment, rats were kept for overnight fasting. Next day forenoon blood was collected from retro-orbital plexus in EDTA-containing tubes. Basal plasma biochemical analysis (‘0’ h) was carried out with auto-blood analyzer instrument (Seimens Dimension Xplus analyser, Newark, USA).

**Experimental design**: Twenty percent fructose and fructose-equivalent sucrose solutions were prepared in distilled water and respective fruit’s juice routinely for oral administration to rats. Rats were grouped as follows:

- Fructose group (Fructose feeding for one month, \( n = 6 \))
- Sucrose group (Sucrose feeding for one month, \( n = 6 \))
- BHF group (Fructose sweetened BH juice for one month, \( n = 6 \))
- SEF group (Fructose sweetened SE juice for one month, \( n = 6 \))

- BHS group (Sucrose sweetened BH juice for one month, \( n = 6 \))
- SES group (Sucrose sweetened SE juice for one month, \( n = 6 \))

Another set of eighteen rats were administered 40% sucrose solution (single dose) daily for three months. Sucrose feeding was withdrawn after three months feeding and rats were sub-divided in following groups:

- Control (normal saline for fifteen days)
- BH (treatment for fifteen days)
- SE (treatment for fifteen days)

A group of normal control animals (treated sham with normal saline) were also maintained throughout experimental period for comparative data analysis.

The dose of juice was decided as reported earlier\(^1\) and administered orally (7.5 mL kg\(^{-1}\) b.wt.) to animals once daily through gastric intubation (between 10-11 AM). Normal rat chow and water was provided ad libitum throughout experimental period. Daily administered dose of fructose and sucrose with and without juice was 1.5 and 3.0 g kg\(^{-1}\) b.wt., respectively.

**Glucose tolerance test**: Fasting blood was collected from retro orbital plexus in EDTA-containing tubes from overnight fasted rats after completion of experiments and plasma glucose levels were analyzed. Oral Glucose tolerance test (OGTT) was conducted by feeding glucose at dose of 2 g kg\(^{-1}\) b.wt. Blood was collected at intervals of 30, 60, 90 and 120th min post-glucose feeding and plasma glucose was analyzed on auto-blood analyzer instrument (Seimens Dimension Xplus analyser, Newark, USA). Two-hour postprandial glycemic load (area under the curve, AUC\(_{0-120 \text{ min}}\) was calculated following trapezoidal rules\(^3\).

**Glycemic load tolerance ability**: Percentage change in glycemic load tolerance ability of rats was calculated as follows:

\[
\frac{\text{AUC}_{\text{fructose 10 days}} - \text{AUC}_{\text{fructose 90 days}}}{\text{AUC}_{\text{fructose 90 days}}} \times 100
\]

AUC\(_{\text{fructose 90 days}}\) represents AUC of rats fed fructose for 90 days and AUC\(_{\text{fructose with glucose}}\) represents AUC of rats of either control or fifteen days juice treated groups after withdrawal of sucrose feeding.

**Measurement of total antioxidant potential**: Total antioxidant potential (TAOP) of plasma was estimated by measuring ABTS\(^+\) cation scavenging activity. Plasma was
RESULTS

Although the yield of juice from both vegetable fruits was similar, concentration of total polyphenols was more than twice and protein more than three times high in SE juice than in BH juice (Table 1). Total flavonoids content was recorded four times more in BH juice than in SE juice. Anthocyanins could not be detectable in these vegetables juice (Table 1).

Plasma glucose level remained high in one month fructose and sucrose fed rats over time. Development of IGT was higher in fructose than in sucrose administered rats. Furthermore, following OGTT, the glucose level in these animals did not return to the level of control group of animals over time (Fig. 1a). However, plasma glucose level returned to the normal level as in control group (120th min) when fructose and sucrose were administered dissolved in BH or SE juice (Fig. 1b, c). Although, the two-hour postprandial glycemic load (AUC$_{0-120}$ min) following OGTT was significantly higher (p<0.05) in fructose or sucrose fed animals with or without juice when compared with control group (Fig. 1d), the BH juice significantly (p<0.05) mitigated glycemic burden in fructose and sucrose administered and SE in sucrose administered rats when compared with sucrose fed rats (Fig. 1d).

One month continuous fructose and sucrose feeding significantly (p<0.05) decreased ABTS$^+$ cation scavenging activity of plasma (Fig. 2a). Drastic decrease in ABTS$^+$ cation scavenging activity was recorded in sucrose-fed animals. Both the vegetables juice significantly prevented decrease in ABTS$^+$ cation scavenging activity induced either by fructose or sucrose except in SEF group (Fig. 2a).

Level of oxidative stress in fructose and sucrose administered-rats was found increased (Fig. 2b). It was significantly (p<0.05) high in rats receiving sucrose with wide individual variations. Moderation in fructose induced oxidative stress was noticed in rats receiving BH and SE juice. Level of sucrose induced oxidative stress was significantly (p<0.05) mitigated (Fig. 2b) by BH (50%) and SE juice (70%).

Three months single-dose daily administration of sucrose solution significantly (p<0.05) increased fasting plasma glucose level of rats (89.7±6.9 vs. 98.2±6.4). Aggravation in IGT following OGTT was observed in
Fig. 1(a-d): Shape of plasma glucose concentration curve and 2 h glycemic load (AUC) following oral glucose tolerance test in rats after one month feeding of fructose, sucrose and, fructose and sucrose-sweetened juice of BH and SE. (a) Shape of plasma glucose concentration curve following fructose and sucrose feeding. (b) Shape of plasma glucose concentration curve following fructose and fructose-sweetened juice feeding. (c) Shape of plasma glucose concentration curve following sucrose and sucrose-sweetened juice feeding and (d) Postprandial glycemic load (AUC_{0-120min}) in different group of experimental animals. *p<0.05 when compared with other groups, **p<0.05 when compared with fructose, a and b p<0.05 when compared with fructose. Values represent Mean±SD, n= 6 animals in each group.

Rats' withdrawn sucrose feeding for 15 days (Fig. 3a). The postprandial glycemic spikes at 30th min in control group of rats were sharper than three months sucrose fed rats. Normalization in glucose tolerance was observed in rats treated with BH and SE juice for 15 days (Fig. 3a). The 2 h glycemic load in three months sucrose-fed rats was significantly higher (p<0.05) than normal rats (Fig. 3b). Three months sucrose feeding (Fig. 3b) induced comparable level of IGT induced by fructose feeding for one month (Fig. 1d). Glycemic load in control group rats was higher (9%) than sucrose fed rats. In comparison with control, BH and SE juice treatment for fifteen days significantly (p<0.05) improved two hour glycemic load (Fig. 3b).

One of the interesting finding in this study was that the glycemic load tolerance ability of rats decreased after withdrawal of sucrose feeding however, BH juice treatment significantly (p<0.05) improved it (Fig. 4). Although SE juice treatment also improved glycemic load tolerance ability of rats; statistical significance could not be observed (Fig. 4).

Decreased plasma TAOP by three months sucrose feeding was found significantly improved after withdrawal of sucrose feeding (Fig. 5a). Treatment of BH and SE juice augmented this improvement. However, statistical significance (p<0.05) could be found with SE juice treatment only. BH and SE juice treatment significantly decreased level of oxidative stress when compared with control (Fig. 5b).

Levels of fluorescent AGEs in plasma and collagen-induced platelet aggregation activity were recorded high in sucrose-fed control group of rats (Fig. 6). In SE juice
Fig. 2(a-b): Plasma total antioxidant potential (TAOP) and level of oxidative stress in rats after one month feeding of fructose, sucrose, and fructose and sucrose-sweetened juice of BH and SE. (a) Plasma total antioxidant potential (TAOP) in various experimental groups, *p<0.05 when compared with sucrose, **p<0.05 when compared with fructose and a and b p<0.05 when compared with control. Values represent Mean±SD, n = 6 animals in each group and (b) Level of oxidative stress in different animal groups, *p<0.05 compared with sucrose, a and b p<0.05 compared with control and fructose, ns: Not significant. Values represent Mean±SD, n = 6 animals in each group.

Fig. 3(a-b): Shape of plasma glucose concentration curve following oral glucose tolerance test and two hour glycemic load (AUC) in rats after three month sucrose feeding followed by BH and SE juice treatment for fifteen days, (a) Shape of plasma glucose concentration curve following oral glucose tolerance test in rats fed sucrose for three months and treated with juices for fifteen days. Values represent mean plasma glucose levels at each time point, n = 5 for normal, 18 for sucrose and 6 for control, BH and SE treated rats and (b) Two hour glycemic load (AUC) in rats of different experimental groups, n = 6 for normal, 18 for sucrose and 6 for control, BH and SE treated rats. *p<0.05 compared with control group, **p<0.05 when compared with normal.

treated animals accumulation of AGEs was found significantly (p<0.05) less (Fig. 6a) however, platelet aggregation activity in whole blood (Fig. 6b) was recorded significantly less (p<0.05) in both the vegetables juice treated rats. SE juice was observed superior than BH juice in reducing both AGEs accumulation and platelet aggregation.

**DISCUSSION**

Plants make mixture of interacting compounds that provide important combination therapies by stimulating multiple therapeutic targets and impart clinical benefits beyond reach of single compound-based drugs. Several mechanisms have been proposed for such therapeutic modalities. Phytochemical components interact with
Fig. 4: Percentage change in glycemic load tolerance ability of rats’ withdrawn sucrose feeding and treated with BH and SE juice for fifteen days. Bar up side on the ‘Y’ axis represents increase and down side represents decrease in glycemic load tolerance ability of rats. Values represent Mean±SD, n=6, *p<0.05 compared with control group.

Fig. 5(a-b): Plasma total antioxidant potential (TAOP) and level of oxidative stress in rats after three month sucrose feeding followed by fifteen days BH and SE juice treatment. (a) Plasma total antioxidant potential (TAOP in terms of % ABTS radical scavenging capacity) of rats under different experimental conditions. **p<0.01 compared with sucrose group and (b) Levels of oxidative stress (ratio of AUC0-10min, mg dl⁻¹ and TAOP) in various experimental groups of rats. **p<0.01 when compared with control group. Values represent Mean±SD, n=6 for normal, 18 for sucrose and 6 for other groups.

various targets to create a combination of effect. One component might alter ability of another to reach its target, components bind separate sites on same target to create a combination effect and increase pharmacological action. Therefore, no single chemical component can be held responsible for activities displayed by complex plant mixtures because combination of whole might display more potential activity than sum of the parts. These fundamentals have been the basis of oriental traditional medicines. In present study, the total polyphenols and protein concentration in SE juice was found more than BH juice whereas total flavonoids content in BH was high than in SE juice. Although, high polyphenolic content in vegetables juice is observed to augment starch induced postprandial glycemia, high protein content in vegetable’s juice is found to reduce starch-induced postprandial glycemic load in rats. Therefore, variations observed in present study with regard to preventive and therapeutic potentials on various parameters might originate with synergistic and or antagonistic effect of different constituents present in juice of different vegetables.

Increased consumption of sugar-sweetened beverages in modern times results due to ample and affordable availability. Sweetened beverages containing high ratio of fructose in particular, have been held responsible for increasing incidences of obesity, alterations in carbohydrate and lipid metabolism and development of T2DM. Our finding that pure-fructose (a monosaccharide) feeding for one month accelerated development of IGT and two hour glycemic load more than feeding of fructose-equivalent sucrose (disaccharide of glucose and fructose) following OGTT finds support with these observations. The reason that fructose is more
Fig. 6: Levels of fluorescent advanced glycation end products (AGE) in glucose intolerant rats treated with BH and SE juice for fifteen days. Data represents Mean ± SD n = 6. (A) *p<0.05 compared with control group. Platelet aggregation activity in whole blood of rats treated with BH and SE juice for fifteen days. Values represent Mean ± SD n = 6 and (B) *p<0.05 compared with control group.

potent in aggravating development of IGT than sucrose may be that as a monosaccharide it is readily absorbed and metabolized by liver. However, when administered in equivalent amount as sucrose, its availability for absorption is delayed because it requires action of disaccharide hydrolyzing enzyme α-glucosidase for its release. Furthermore, fructose and glucose follows different metabolic pathways. While glucose metabolism is regulated by main regulatory step of glycolysis controlled by phosphofructokinase, fructose bypasses this regulatory step and can continuously enter glycolytic pathway and adds to the uncontrolled production of glucose. Furthermore, though fructose and sucrose solutions decrease ability of animals to tolerate glucose load, influence of fructose has been reported more than glucose. Long terms sucrose feeding (three months) induced development of IGT up to the level induced by fructose in one month along with appearance of Impaired Fasting Glycemia (IFG). Interestingly, withdrawal of sucrose after three months worsened glycemic load tolerance ability of rats without any treatment. These findings warrant sudden withdrawal of sugar intake without any precautionary measures.

Shape of plasma glucose concentration curve during OGTT has been recognized as an important measure to predict future risk of T2DM development. It has been demonstrated that subjects with normal glucose tolerance and IFG, whose plasma glucose concentration does not return to baseline after 60 min following OGTT, possess significantly higher risk of T2DM development. Similar shape of plasma glucose curve following OGTT was observed in fructose and sucrose fed rats and demonstrates their IGT inducing potential. It was interesting to note that juice of both the vegetable fruits mitigated IGT inducing potential of fructose and sucrose. BH juice was more potent than SE in reducing fructose induced IGT development. Mechanism by which BH juice reduces fructose induced IGT development requires further investigation.

IGT and IFG represent intermediate state, which often progresses to overt diabetes within few year and is defined as pre-diabetes. Pre-diabetes may be associated with increased risk of microvascular and macrovascular complications development. Thus, reverting pre-diabetes state and preventing its development in to diabetes presents mammoth concern. Three months sucrose feeding to rats induced both IFG and IGT. Fifteen days treatment of these rats with vegetables juice normalized shape of plasma glucose curve following OGTT and two hour glycemic load where as it remained higher in rats without any treatment. BH juice treatment was found significantly potent in improving glycemic load tolerance ability of rats.

Investigations report that fructose acts as pro-oxidant and induces oxidative stress by decreasing antioxidant defense and increasing free radicals generation. However, glucose has been observed to act as pro-oxidant by enhancing pro-oxidant enzyme's activity. ABTS•⁺ cation has been successfully utilized to determine free radical scavenging/antioxidant potential of plasma. Utilizing ABTS•⁺ cation scavenging activity as a measure of plasma TAOP; we found that both fructose and sucrose significantly decreased plasma TAOP, sucrose being more potent than fructose. The intense decrease in antioxidant potential of plasma by sucrose may be due to cumulative pro-oxidant and oxidative stress inducing effect of fructose and glucose units present in disaccharide. Both the vegetable's juice offered significant protection against sucrose induced decrease in plasma TAOP. SE offered better protection than BH. BH mitigated fructose induced decrease in plasma TAOP significantly. Withdrawal of sucrose
feeding significantly improved plasma TAOP. Although, more increase in plasma TAOP by BH and SE treatment was noticed it was not significantly higher than untreated control rats. It still remains an open question that withdrawal of sucrose feeding decreased glycemic load tolerance ability of rats, TAOP of animals increased significantly.

Postprandial period is a pro-oxidant state. Postprandial period is also a time of active oxidative metabolism and generation of free radicals. Generation of pro-oxidant environment or decrease in antioxidant defense represents level of oxidative stress. An imbalance between oxidants generation and antioxidant defense in favor of oxidants potentially leading to physiological dysfunction and/or biomolecular damage is referred to as oxidative stress. Utilizing these parameters, for example, two hour glycemic load as a state inducing pro-oxidant environment and TAOP as available antioxidant defense, we obtained oxidative stress level in experimental animals. Oxidative stress level in sucrose-fed rats was moderately high than control group rats however; it was significantly high in sucrose-fed animals. These results are in accordance with the earlier observations that sucrose-rich diet induces moderate oxidative stress. BH and SE juice moderated development of oxidative stress induced by sucrose however, offered significant protection against sucrose induced oxidative stress. Similar results were obtained when rats were fed sucrose for three months. Both BH and SE juice treatment significantly accelerated decrease in oxidative stress level than rats treated as placebo.

Hyperglycemia is known to potentiate platelet activation and increase generation of advanced glycation end products (AGEs). These processes are further amplified under oxidative stress conditions. Additionally, increased concentrations of AGEs further exacerbate platelet aggregation. Altered platelet function is prevalent in diabetes and participates in pathogenesis of diabetic vascular complications by promoting microthrombus formation. Both of these abnormalities were detected in three months sucrose fed rats. Therapies possessing antihyperglycemic and antioxidative properties therefore might reduce development of these complications. Whole blood platelet aggregation assay is a model more closely related to physiological conditions. Therefore, inhibition of whole blood aggregation would suggest antithrombotic potential of a therapy. Juice of BH and SE significantly reduced collagen induced platelet aggregation in hyperglycemic blood. SE juice was found better in reducing collagen induced platelet aggregation and AGEs formation than BH juice. Presence of both antihyperglycemic and antioxidative activities in juice of BH and SE may be responsible for antiplatelet aggregation and anti-AGEs activities in this study.

CONCLUSION
Sugar-sweetened beverages are potent inducer of IGT, IFG, oxidative stress and risk factor responsible for development of hyperglycemia induced complications. Juice of BH and SE may offer protective and therapeutic measures against physiological and biochemical imbalances induced by increased intake of sugar-sweetened beverages by virtue of presence of multiple preventive and therapeutic activities. This report provides for the first time evidence that consumption of raw juice from vegetables may help ameliorate sweetened beverages induced metabolic disturbances and impart health benefits in IGT individuals.

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