RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2013.197.207

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

[1]Ashok Kumar Tiwari, [1]Iragavarapu Anusha, [1]Maddineni Sumangali, [1]Domati Anand Kumar, [1]Kuncha Madhusudana and [1,2]Sachin Bharat Agawane

[1]Medicinal Chemistry and Pharmacology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad-500607, India

[2]Biochemical 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 juice of BH and 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

Pharmacologia 4 (3): 197-207, 2013

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[1,2,3,4]. T2DM is emerging at epidemic rate in many parts of the world including developed nations like United States of America[5] and developing countries like China[6] and India[7]. 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[1]. 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[8]. Increased amount of fructose in diet is identified as an important causative factor responsible for increasing insulin resistance,

Corresponding Author: Ashok K. Tiwari, Medicinal Chemistry and Pharmacology Division, CSIR-Indian Institute of Chemical Technology, Hyderabad-500607, India Tel:+91-040-27191875 Fax:+91-040-27193189

© 2013 Science Reuters, UK

decreasing insulin sensitivity and consequently, development of Impaired Glucose Tolerance[2] (IGT). High-fructose diet is reported to induce oxidative stress by decreasing antioxidant defense[9] and increasing free radicals generation[10] Furthermore, postprandial hyperglycemic excursions (PPHGE) and hyperglycemic spikes even in non-diabetic individuals also increase oxidative stress[11,12]. Postprandial hyperglycemic spikes instigate redox imbalance in short term and lead to the development of complex chronic diseases in long-term exposure[13].

Fruits and vegetables are being identified as potential readily available and cost-effective resources for development of new generation therapeutics for lifestyle related disorders[14,15]. 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[16,17] However, health benefits of polyphenols-rich dietary supplements[18], dietary antioxidant supplements[19] and antioxidant compounds from natural resources[20] 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[21] 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[22,23,24]. Although, uric acid possesses antioxidant properties, it is also a risk factor for several diseases[25]. In addition, some antioxidant-rich fruits augment hyperlipidemia[26] and antioxidant-rich fraction of some cereals[27] and dietary formulations[28] display proglycemic activity. Vegetables are reported to be rich source of biological antioxidants[17] and serve cost effective culinary item. However, high polyphenolic content in vegetable's juice was observed recently to augment starch induced PPHGE[15].

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 *Sechium edule* (SE) was identified to mitigate starch induced PPHGE in rats[15]. Although, BH is extensively used in traditional oriental medicine for treatment of gastrointestinal problems, respiratory, heart and urinary diseases[29] and SE advocated for treatment of arteriosclerosis, hypertension and inflammation[30,31], 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'-Azinobis-3-ethyl benzthiazoline-6-sulphonic 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* Thunb. Cogn. (Family Cucurbitaceae) and *Sechium 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[32]. 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_2Co_3 (20% w/v). Mixture was incubated at room temperature (60 min). Absorbance (765 nm) was recorded spectrophotometrically on microplate reader (SpectraMax plus[384], Molecular Devices, Sunnyvale, CA). Total polyphenolic content was expressed as milligrams of Gallic Acid Equivalent (GAE)/mL juice
- **Total anthocyanins:** Anthocyanins in juice were determined as described by Giusti *et al.*[33]. To 25 mM potassium chloride solution (pH 1.0) and

© 2013 Science Reuters, UK

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 = $[(A_{510}-A_{700})$pH 1.0-$(A_{510}-A_{700})$ pH 4.5] 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% $AlCl_3.6H_2O$ in a 96 well micro plate[34]. 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[35]. Briefly, 10 µLjuice was mixed with 240 µL of Bradford reagent and absorbance was read at 595 nm spectrophometrically. 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 polyvinyl 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[15] 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, U.S.A.). Two-hour postprandial glycemic load (area under the curve, $AUC_{0\text{-}120\ min}$) was calculated following trapezoidal rules[15].

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

$$\frac{AUC_{sucrose\ 90\ days}\text{-}AUC_{sucrose\ \ with\ drawn}}{AUC_{sucrose\ 90\ days}}\times 100$$

$AUC_{sucrose\ 90\ days}$ represents AUC of rats fed sucrose for 90 days and $AUC_{sucrose\ with\ drawn}$ 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 $ABTS^{\bullet+}$ cation scavenging activity. Plasma was

© 2013 Science Reuters, UK

deproteinised[22] by mixing equal volume of acetonitrile and incubated for 2 min at 20°C and centrifuged for 10 minutes (9500×g, 4°C). $ABTS^{\bullet+}$ cation scavenging potential in deproteinised plasma was measured as reported earlier with slight modification[15]. Briefly, 100 mL stock solution of $ABTS^{\bullet+}$ (0.5 mM) was prepared by addition of 1 mL potassium persulfate [6.89 mM in PBS (pH 8.0)]. Mixture was stored in dark for 16 h. Deproteinised plasma (10 µL) was mixed with 190 µL of $ABTS^{\bullet+}$ in a 96-well microplate. Absorbance of decolorized $ABTS^{\bullet+}$ was measured at 734 nm after 10 min incubation in the dark on a microplate reader. Percentage $ABTS^{\bullet+}$ scavenging was calculated by applying following formula:

$$\frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Determination of level of oxidative stress: Considering two-hour postprandial glycemic load ($AUC_{0\text{-}120\ min}$) as metabolic/oxidative stress inducer (prooxidant state) and plasma TAOP as available antioxidant defense, the ratio of $AUC_{0\text{-}120\ min}$ and TAOP was calculated ($AUC_{0\text{-}120\ min}$/TAOP) as a measure representing level of oxidative stress in each group.

Analysis of whole blood platelet aggregation activity: Whole blood platelet aggregation activity was measured by impedance method[36]. Blood was collected in tubes containing 3.8% sodium citrate. One milliliter whole blood (diluted 1:1 with physiological saline) was transfer to plastic cuvette pre-heated at 37°C. Teflon-coated bar was added and electrode was placed in cuvette. Aggregation was induced by collagen (10 µg mL^{-1}) and maximum amplitude was recorded on platelet aggregometer (Chronolog-Corporation USA, Model 700). Platelet aggregation was quantified as change in impedance and expressed in ohms.

Estimation of fluorescent AGEs: Plasma was deproteinised by mixing with equal volume of acetonitrile, incubated for 2 min (20°C) and centrifuged for 10 minutes (9500 x g, 4°C). Fluorescent AGEs in supernatant were assayed[37] (excitation 370 nm, emission 440 nm) and expressed in fluorescent units.

Statistical analysis: One-way ANOVA followed by Bonferroni's multiple comparison tests was applied to compare difference in study groups. Differences between pre-and post treatments were analyzed following Mann Whitney (two-tailed) test. Criterion for statistical significance was $p<0.05$. Statistical analysis was performed using GraphPad PRISM® Version 5.01 (GraphPad Software, Inc. California, USA) software.

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 ($AUC_{0\text{-}120\ min}$) 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 fructose fed rats (Fig. 1d).

One month continuous fructose and sucrose feeding significantly ($p<0.05$) decreased $ABTS^{\bullet+}$ cation scavenging activity of plasma (Fig. 2a). Drastic decrease in $ABTS^{\bullet+}$ cation scavenging activity was recorded in sucrose-fed animals. Both the vegetables juice significantly prevented decrease in $ABTS^{\bullet+}$ 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

Table 1: Chemical components analyzed in juices

	% yield (mL/100 g)	TPP (µg GAE/mL)	TF (µg RE/mL)	TA (% w/w)	TP (µg BSAE/mL)
BH	68.96±0.5	140±50	10.00±0.40	ND	390±20
SE	67.34±0.4	320±60	2.50±0.10	ND	1380±30

BH:*Benincasa hispida*, SE: *Sechium edule*, TPP: Total polyphenols, GAE: Gallic acid equivalent, TF: Total flavonoids, RE: Rutin equivalent, TA: Total anthocyanins, TP: Total protein, BSAE: Bovine serum albumin equivalent. ND: Not detected, Values represent Mean±SD, n = 8-10 measurements

© 2013 Science Reuters, UK

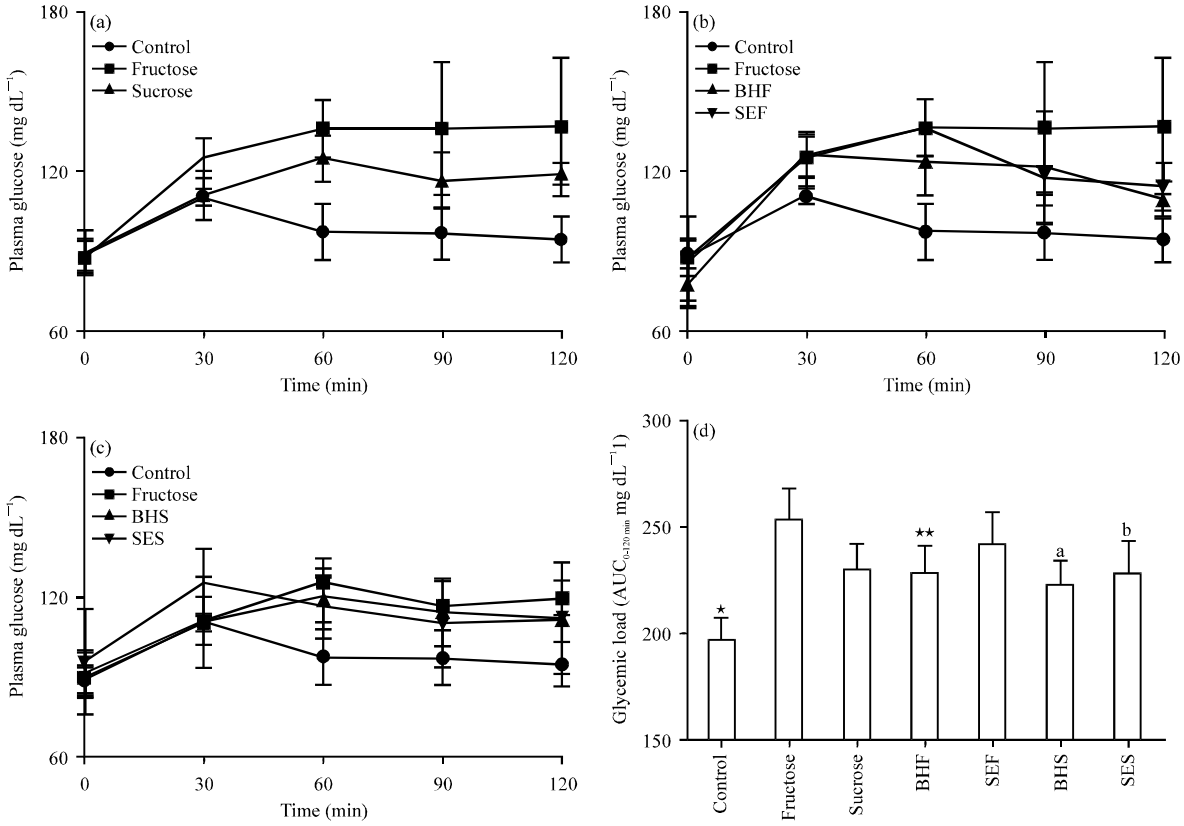

Fig. 1(a-d): Shape of plasma glucose concentration curve and 2 h glycemic load (AUC) following oral glucose tolerance test in rats after one month feeding of fructose, sucrose and, fructose and sucrose-sweetened juice of BH and SE, (a) Shape of plasma glucose concentration curve following fructose and 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 ($AUC_{0\text{-}120\,min}$) 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

© 2013 Science Reuters, UK

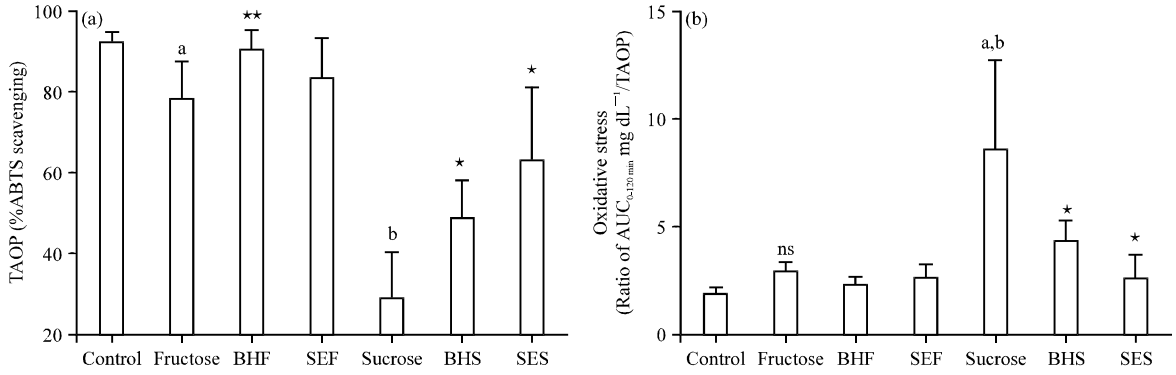

Fig. 2(a-b): Plasma total antioxidant potential (TAOP) and level of oxidative stress in rats after one month feeding of fructose, sucrose and, fructose and sucrose-sweetened juice of BH and SE, (a) Plasma total antioxidant potential (TAOP) in various experimental groups, *p<0.05 when compared with sucrose, **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

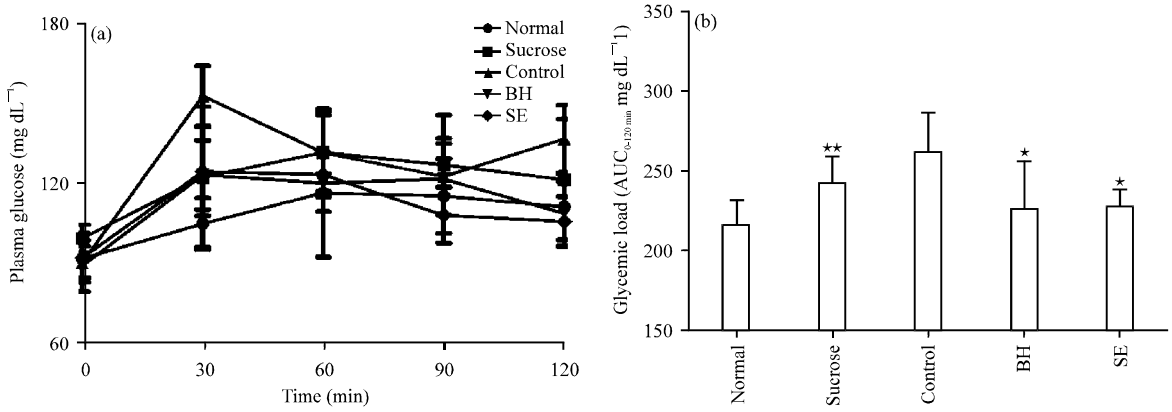

Fig. 3(a-b): Shape of plasma glucose concentration curve following oral glucose tolerance test and two hour glycemic load (AUC) in rats after three month sucrose feeding followed by BH and SE juice treatment for fifteen days, (a) Shape of plasma glucose concentration curve following oral glucose tolerance 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 = 6 for normal, 18 for sucrose and 6 for control, BH and SE treated rats and (b) Two hour glycemic load ($AUC_{0\text{-}120\ min}$ mg dL^{-1}) 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[38]. Several mechanisms have been proposed for such therapeutic modalities. Phytochemical components interact with

© 2013 Science Reuters, UK

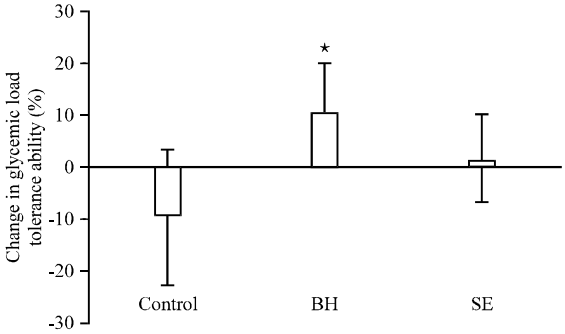

Fig. 4: Percentage change in glycemic load tolerance ability of rats' withdrawn sucrose feeding and treated with BH and SE juice for fifteen days. Bar up side on the 'Y' axis represents increase and down side represents decrease in glycemic load tolerance ability of rats. Values represent Mean±SD, n = 6, *p<0.05 compared with control group

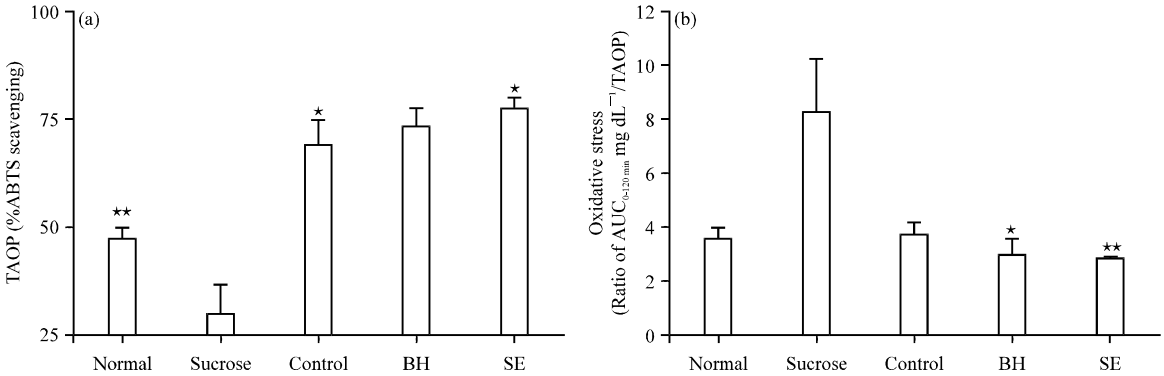

Fig. 5(a-b): Plasma total antioxidant potential (TAOP) and level of oxidative stress in rats after three month sucrose feeding followed by fifteen days BH and SE juice treatment, (a) Plasma total antioxidant potential (TAOP in terms of % ABTS radical scavenging capacity) of rats under different experimental conditions. *,**p<0.05 compared with sucrose group and (b) Levels of oxidative stress (ratio of $AUC_{0\text{-}120\,min}$ mg dL^{-1} and TAOP) in various experimental groups of rats. *,**p<0.05 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[39]. 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[15]. 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[40], alterations in carbohydrate and lipid metabolism[41] and development of T2DM[2]. 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

© 2013 Science Reuters, UK

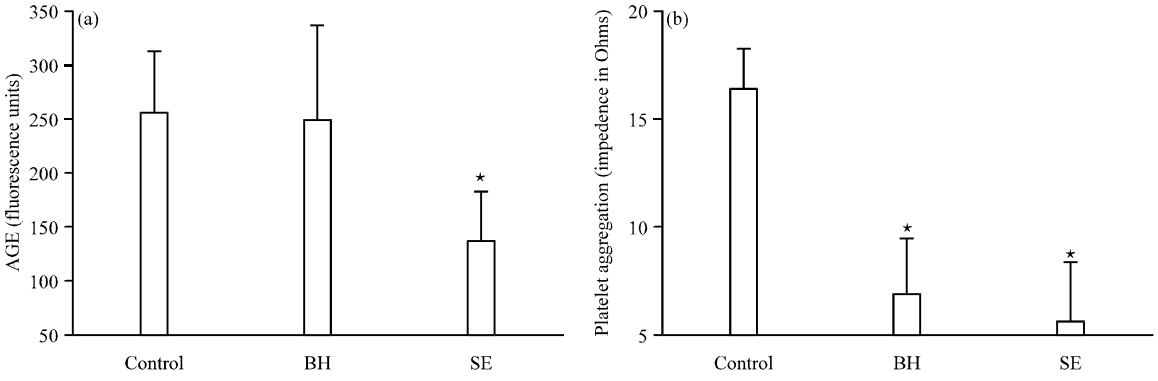

Fig. 6: Levels of fluorescent advanced glycation end products (AGE) in glucose intolerant rats treated with BH and SE juice for fifteen days. Data represents Mean±SD n = 6. (A) *p<0.05 compared with control group. Platelets aggregation activity in whole blood of rats treated with BH and SE juice for 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[42]. 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 by-passes this regulatory step and can continuously enter glycolytic pathway and adds to the uncontrolled production of glucose[42]. Furthermore, though fructose and sucrose solutions decrease ability of animals to tolerate glucose load, influence of fructose has been reported more than glucose[43,44]. 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 feeding 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[45]. 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[45]. Pre-diabetes may be associated with increased risk of microvascular and macrovascular complications development[45]. Thus, reverting pre-diabetes state and preventing its development in to diabetes presents mammoth concern[45]. 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[10,46] and induces oxidative stress by decreasing antioxidant defense and increasing free radicals generation[10]. However, glucose has been observed to act as pro-oxidant by enhancing pro-oxidant enzyme's activity[47]. $ABTS^{\bullet+}$ cation has been successfully utilized to determine free-radical scavenging/antioxidant potential of plasma[48]. Utilizing $ABTS^{\bullet+}$ 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

© 2013 Science Reuters, UK

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[21]. 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 fructose-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 fructose-rich diet induces moderate oxidative stress[49].BH and SE juice moderated development of oxidative stress induced by fructose however, offered significant protection against sucrose induced oxidative stress development. 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[50] and increase generation of advanced glycation end products (AGEs). These processes are further amplified under oxidative stress conditions[12]. Additionally, increased concentrations of AGEs further exacerbate platelet aggregation[51]. Altered platelet function is prevalent in diabetes and participates in pathogenesis of diabetic vascular complications by promoting microthrombus formation[52].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[36]. 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.

ACKNOWLEDGMENTS

Authors thank D'IICT for constant support and encouragement. This research was supported in part from Grant MLP-0001 (CSIR, New Delhi). Authors declare no conflict of interest, financial or otherwise.

REFERENCES

1. Malik, V.S., B.M. Papopkin, G.A. Bray, J.P. Despres, W.C. Willett and F.B. Hu, 2010. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes: A meta-analysis. Diabetes Care, 33: 2477-2483.
2. Malik, V.S., B.M. Papopkin, G.A. Bray, J.P. Despres and F.B. Hu, 2010. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus and cardiovascular disease risk. Circulation, 121: 1356-1364.
3. Misra, A., N. Singhal, B. Sivakumar, N. Bhagat, A. Jaisawal and L. Khurana, 2011. Nutrition transition in India: Secular trends in dietary intake and their relationship to diet-related non-communicable diseases. J. Diabetes, 3: 278-292.
4. Hu, E.A., A. Pan, V. Malik and Q. Sun, 2012. White rice consumption and risk of type 2 diabetes: Meta-analysis and systematic review. BMJ., Vol. 344. 10.1136/bmj.e1454
5. Shaw, J.E., R.A. Sicree and P.Z. Zimmet, 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pract., 87: 4-14.
6. Zhao, D., F. Zhao, Y. Li and Z. Zeng, 2012. Projected and observed diabetes epidemics in China and beyond. Curr. Cardiol. Rep., 14: 106-111.
7. Anjana, R.M., R. Pradeepa, M. Deepa, M. Datta and V. Sudha *et al.*, 2011. Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: Phase I results of the Indian Council of Medical Research-India Diabetes (ICMR-INDIAB) study. Diabetologia, 54: 3022-3027.
8. Williamson, G., 2013. Possible effects of dietary polyphenols on sugar absorption and digestion. Mol. Nutr. Food Res., 57: 48-57.

© 2013 Science Reuters, UK

9. Busserolles, J., E. Gueux, E. Rock, A. Mazur and Y. Rayssiguier, 2002. Substituting honey for refined carbohydrates protects rats from hypertriglyceridemic and prooxidative effects of fructose. J. Nutr., 132: 3379-3382.
10. Busserolles, J., E. Gueux, E. Rock, A. Mazur and Y. Rayssiguier, 2003. High fructose feeding of magnesium deficient rats is associated with increased plasma triglyceride concentration and increased oxidative stress. Magnesium Res., 16: 7-12.
11. O'Keefe, J.H., N.M. Gheewala and J.O. O'Keefe, 2008. Dietary strategies for improving post-prandial glucose, lipids, inflammation and cardiovascular health. J. Am. Coll. Cardiol., 51: 249-255.
12. Giacco, F., M. Brownlee and A.M. Schmidt, 2010. Oxidative stress and diabetic complications. Circ. Res., 107: 1058-1070.
13. Kim, J.Y. and O. Kwo, 2011. Culinary plants and their potential impact on metabolic overload. Ann. N. Y. Acad. Sci., 1229: 133-139.
14. Parmar, H.S., Y. Dixit and K. Anand, 2010. Fruit and vegetable peels: Paving the way towards the development of new generation therapeutics. Drug Discov. Ther., 4: 314-325.
15. Tiwari, A.K., K.S. Reddy, J. Radhakrishnan, D.A. Kumar, A. Zehra, S.B. Agawane and K. Madhusudana, 2011. Influence of antioxidant rich fresh vegetable juices on starch induced postprandial hyperglycemia in rats. Food Funct., 2: 521-528.
16. Wang, S., J.P. Melnyk, R. Tsao and M.F. Marcone, 2011. How natural dietary antioxidants in fruits, vegetables and legumes promote vascular health. Food Res. Int., 44: 14-22.
17. Wooton-Beard, P.C., A. Moran and L. Ryan, 2011. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP, DPPH, ABTS and folin-ciocalteu methods. Food Res. Int., 44: 217-221.
18. Martin, K.R. and C.L. Appel, 2010. Polyphenols as dietary supplements: A double-edged sword. Nutr. Dietary Suppl., 2: 1-12.
19. Serrano, J., A. Cipak, J. Boada, H. Gonzalo and D. Cacabelos *et al*., 2010. Double-edged sword behaviour of gallic acid and its interaction with peroxidases in human microvascular endothelial cell culture (HMEC-1) Antioxidant and pro-oxidant effects. Acta Biochem. Pol., 57: 193-198.
20. Bouayed, J. and T. Bohn, 2010. Exogenous antioxidants: Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses. Oxidative Med. Cell. Longevity, 3: 228-237.
21. Burton-Freeman, B., 2010. Postprandial metabolic events and fruit-derived phenolics: A review of the science. Br. J. Nutr., 104: S1-S14.
22. Godycki-Cwirko, M., M. Krol, B. Krol, A. Zwolinska and K. Kolodziejczyk *et al*., 2010. Uric acid but not apple polyphenols is responsible for the rise of plasma antioxidant activity after apple juice consumption in healthy subjects. J. Am. Coll. Nutr., 29: 397-406.
23. Lolito, S.B. and B. Frei, 2004. The increase in human plasma antioxidant capacity after apple consumption is due to the metabolic effect of fructose on urate, not apple derived antioxidants and flavonoids. Free Radical Biol. Med., 37: 251-258.
24. Zawiasa, A., M. Szklarek-Kubicka, J. Fijalkowska-Morawska, D. Nowak, J. Rysz, B. Mamelka and M. Nowicki, 2009. Effect of oral fructose load on serum uric acid and lipids in kidney transplant recipients treated with cyclosporine or tacrolimus. Transplant Proc., 41: 188-191.
25. Jin, M., F. Yang, I. Yang, Y. Yin, J.J. Luo, H. Wang and X.F. Yang, 2012. Uric acid, hyperuricemia and vascular diseases. Front. Biosci., 17: 656-669.
26. Alvarez-Parrilla, E., L.A. De La Rosa, P. Legarreta, L. Saenz, J. Rodrigo-Garcia and G.A. Gonzalez-Aguilar, 2010. Daily consumption of apple, pear and orange juice differently affects plasma lipids and antioxidant capacity of smoking and non-smoking adults. Int. J. Food Sci. Nutr., 61: 369-380.
27. Tiwari, A.K., M. Swapna, S.B. Ayesha, A. Zehra, S.B. Agawane and K. Madhusudhana, 2011. Identification of proglycemic and antihyperglycemic activity in antioxidant rich fraction of some common food grains. Int. Food Res. J., 18: 915-923.
28. Tiwari, A.K., M.P. Kumar, D.A. Kumar, S.B. Agawane, K. Madhusudana and A. Zehra, 2012. Ayurvedic dietary formulations and postprandial glycemia in rats. Int. Food Res. J., 19: 765-773.
29. Asolkar, L.V., K.K. Kakkar and O.J. Chakre, 1992. Second Supplement to Glossary of Indian Medicinal Plants with Active Principles: Part-1 (A-K), (1965-1981), Volume 1. Publications and Information Directorate, New Delhi, ISBN: 9788172360481, Pages: 414.
30. Flores, E.M., 1989. The chayote, Sechium edule Swartz (Cucurbitaceae). Rev. Biol. Trop., 1: 1-54.
31. Riberio, R.D.A., F. de Barros, M.M.R. Fiuza de Melo, C. Muniz and S. Chieia *et al*., 1988. Acute diuretic effects in conscious rats produced by some medicinal plants used in the state of Sao Paulo, Brasil. J. Ethnopharmacol., 24: 19-29.
32. Yao, Y., W. Sang, M. Zhou and G. Ren, 2010. Antioxidant and α-glucosidase inhibitory activity of colored grains in China. J. Agric. Food Chem., 58: 770-774.

© 2013 Science Reuters, UK

33. Giusti, M.M., L.E. Rodriguez-Saona and R.E. Wrolstad, 1999. Molar absorptivity and color characteristics of acylated and non-acylated pelargonidin-based anthocyanins. J. Agric. Food Chem., 47: 4631-4637.
34. Hsieh, C.Y and S.T. Chang, 2010. Antioxidant activities and xanthine oxidase inhibitory effects of phenolic phytochemicals from Acacia confusa twigs and branches. J. Agric. Food Chem., 58: 1578-1583.
35. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
36. Brito, F.C.F., A.E. Kummerle, C. Lugnier, C.A.M. Fraga, E.J. Barreiro and A.L.P. Miranda, 2010. Novel thienylacyl hydazone derivatives inhibit platelet aggregation through cyclic nucleotides modulation and thromboxane A_2 synthesis inhibition. Eur. J. Pharmacol., 638: 5-12.
37. Forbes, J.M., L.T. Yee, V. Thallas, M. Lassila and R. Candido *et al.*, 2004. Advanced glycation end products interventions reduce diabetes accelerated atherosclerosis. Diabetes, 53: 1813-1823.
38. Schmidt, B.M., D.M. Ribnicky, P.E. Lipsky and I. Raskin, 2007. Revisiting the ancient concept of botanical therapeutics. Nat. Chem. Biol., 3: 360-366.
39. Zimmermann, G.R., J. Lehar and C.T. Keith, 2007. Multi-target therapeutics: When the whole is greater than the sum of the parts. Drug Discov. Today, 12: 34-42.
40. Bray, G.A., S.J. Nielsen and B.M. Popkin, 2004. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am. J. Clin. Nutr., 79: 537-543.
41. Havel, P.J., 2005. Dietary fructose: Implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. Nutr. Rev., 63: 133-157.
42. Kanarek, R.B. and N. Orthen-Gambill, 1982. Differential effects of sucrose, fructose and glucose on carbohydrate-induced obesity in rats. J. Nutr., 112: 1546-1554.
43. Levine, R., 1986. Monosaccharides in health and disease. Annu. Rev. Nutr., 6: 211-224.
44. Abdul-Ghani, M.A., V. Lyssenko, T. Tuomi, R.A. DeFronzo and L. Groop, 2010. The shape of plasma glucose concentration curve during OGTT predicts future risk of type 2 diabetes. Diabetes/Metab. Res. Rev., 26: 280-286.
45. Busserolles, J., W. Zimowska, E. Rock, Y. Rayssiguier and A. Mazur, 2002. Rats fed a high sucrose diet have altered heart antioxidant enzyme activity and gene expression. Life Sci., 71: 1303-1312.
46. Bayraktutan, U., P. Weidig, D. McMaster and S. Ulker, 2004. High glucose mediates pro-oxidant and antioxidant enzyme activities in coronary endothelial cells. Diabet. Obes. Metab., 6: 432-441.
47. Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26: 1231-1237.
48. Francini, F., M.C. Castro, G. Schinella, M.E. Garcia and B. Maiztegui *et al.*, 2010. Changes induced by a fructose-rich diet on hepatic metabolism and the antioxidant system. Life Sci., 86: 965-971.
49. Kakouros, N., J.J. Rade, A. Kourliouros and J.R. Resar, 2011. Platelet function in patients with diabetes mellitus: From a theoretical to a practical perspective. Int. J. Endocrinol., 10.1155/2011/742719
50. Zhu, W., W. Li and R.L. Silverstein, 2012. Advanced glycation end products induce a prothrombotic phenotype in mice via interaction with platelet CD36. Blood, 119: 6136-6144.
51. Randriamboavonjy, V. and I. Fleming, 2012. Platelet function and signaling in diabetes mellitus. Curr. Vasc. Pharmacol., 10: 532-538.
52. Basciano, H., L. Federico and K. Adeli, 2005. Fructose, insulin resistance and metabolic dyslipidemia. Nutr. Metab., Vol. 2. 10.1186/1743-7075-2-5

© 2013 Science Reuters, UK