XYLITOL IMPROVES ANTI-OXIDATIVE DEFENSE SYSTEM IN SERUM, LIVER, HEART, KIDNEY AND PANCREAS OF NORMAL AND TYPE 2 DIABETES MODEL OF RATS

CHIKA IFEANYI CHUKWUMA and MD. SHAHIDUL ISLAM*

Department of Biochemistry, School of Life Sciences, University of KwaZulu-Natal (Westville Campus), Durban 4000, South Africa

Abstract: The present study investigated the anti-oxidative effects of xylitol both *in vitro* and *in vivo* in normal and type 2 diabetes (T2D) rat model. Free radical scavenging and ferric reducing potentials of different concentrations of xylitol were investigated *in vitro*. For *in vivo* study, six weeks old male Sprague-Dawley rats were divided into four groups, namely: Normal Control (NC), Diabetic Control (DBC), Normal Xylitol (NXYL) and Diabetic Xylitol (DXYL). T2D was induced in the DBC and DXYL groups. After the confirmation of diabetes, a 10% xylitol solution was supplied instead of drinking water to NXYL and DXYL, while normal drinking water was supplied to NC and DBC *ad libitum*. After five weeks intervention period, the animals were sacrificed and thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) concentrations as well as superoxide dismutase, catalase glutathione reductase and glutathione peroxidase activities were determined in the liver, heart, kidney, pancreatic tissues and serum samples. Xylitol exhibited significant (p < 0.05) *in vitro* nitric oxide and hydroxyl radical scavenging and ferric reducing activities. *In vivo* study revealed significant (p < 0.05) reduction in TBARS concentrations in the xylitol consuming groups compared to their respective controls. Significant (p < 0.05) increase in GSH levels and antioxidant enzyme activities were observed in analyzed tissues and serum of xylitol-fed animals compared to their respective controls. Results of this study indicate that xylitol has strong anti-oxidative potential against T2D-associated oxidative stress. Hence, xylitol can be used as a potential supplement in diabetic foods and food products.

Keywords: xylitol, antioxidants, oxidative stress, sugar substitute, type 2 diabetes

Abbreviations: AA - ascorbic acid, CAT - catalase, DBC- diabetic control, DXYL- diabetic xylitol, GA - gallic acid, GPx - glutathione peroxidase, GR - glutathione reductase, GSH - reduced glutathione, MDA - malondialdehyde, NC - normal control, NXYL - normal xylitol, ROS - reactive oxygen species, SOD - superoxide dismutase, TBARS - thiobarbituric acid reactive substances, T2D - type 2 diabetes

Diabetes is a major threat to global public health when type 2 diabetes (T2D) has been described as the most prevalent form of diabetes. It is characterized by insulin resistance, partial pancreatic beta-cell dysfunction accompanied with hyperglycemia (1, 2). Hyperglycemia–induced oxidative stress has been implicated in the symptoms, progression and complications of diabetes (2, 3). Persistent hyperglycemia is also responsible for elevated levels of mitochondrial energy production, glucose oxidation, protein glycosylation and lipid peroxidation leading to increase production of free radicals and reactive oxygen species (ROS) (3, 4). Oxidative stress sets in when the generated free radicals surpasses the body's antioxidant defense system (4).

Sugar alcohols are known for their effects on the integrated blood glucose response compared to sucrose; thus, are used as sugar replacers or sweeteners by both normoglycemic and diabetic individuals. Among other sugar alcohols, xylitol has been extensively studied, showing numerous beneficial effects and potential clinical uses other than being used as a sweetener. Consistent results from different *in vitro* and clinical studies have confirmed that xylitol possesses anti-cariogenic and remineralization properties (5). Some recent studies reported the ability of xylitol to reduce non-fasting blood glucose and serum fructosamine levels, increase serum insulin level, and improve glucose tolerance ability in normal (6) and type 2 diabetic rats (7) compared to their respective controls. Another recent study

---

reported that a bolus dose of xylitol (1 g/kg body weight) inhibited small intestinal absorption of glucose in normal and diabetic rats (8). Two more recent studies reported that xylitol improved pancreatic islet morphology in type 2 diabetic rats (9) and prevented free fatty acid-induced insulin resistance in normal rats (10). Additionally, an old study demonstrated that xylitol decreased oxidized and reduced glutathione ratio *in vitro* (11). Some recent *in vitro* studies have also reported the higher antioxidant activities of xylitol compared to some commercially available sweeteners (12, 13). Additionally, another *in vivo* study suggests that 10% xylitol reduced non enzymatic glycosylation of acid-soluble collagen in diabetic rats (14), which has been reported to be elevated in insulin-dependent diabetic patients (15). However, the effect of xylitol on *in vivo* anti-oxidative defense system either in normal or in diabetic condition is still unknown.

Considering the above statements and the significant role of oxidative stress in the progression and complications of diabetes, the present study was conducted to investigate the effects of xylitol on *in vitro* anti-oxidant activity and *in vivo* anti-oxidative defense system both in normal and type 2 diabetic rat model.

MATERIALS AND METHODS

Chemicals and reagents

Pure xylitol was kindly donated by a local South African company, Sweet Nothings (Pietermaritzburg, South Africa). Food grade fructose was purchased from a local supplier (Polychem Supplies CC., Durban, South Africa). Mono-basic sodium phosphate, sodium lauryl sulfate, acetic acid, thiobarbituric acid, malondialdehydebis-(dimethylacetal), mono-basic potassium phosphate, hydrogen peroxide, perchloric acid, EDTA disodium, and sodium hydroxide pellets were purchased from Merck, South Africa. Dibasic sodium phosphate, dibasic potassium phosphate, 2-deoxy-D-ribose, triton X-100, 6-hydroxy-dopamine, diethylenetriamine – pentaacetic acid , glutathione reductase (GR), sodium azide, Tris-HCl, L-glutathione oxidized, NADPH, trichloroacetic acid (TCA), Griess reagent, sodium nitroprusside, potassium ferric cyanide, ferric chloride, ascorbic acid (AA), gallic acid (GA), sodium bicarbonate, reduced glutathione (GSH), 5,5'-dithiobis-(2-nitrobenzoic acid) and *tert*-butyl hydroperoxide were purchased from Sigma Aldrich, Germany.

In vitro study
Hydroxyl radical (OH·) scavenging assay

The hydroxyl radical (OH·) scavenging activity of xylitol was determined using a non-site-specific hydroxyl radical-mediated 2-deoxy-D-ribose degradation method (16). Briefly, a 200 µL of premixed 100 µM $FeCl_3$ and 100 µM EDTA (1 : 1 v/v) solution, 100 µL of 10 mM H_2O_2, 360 µL of 10 mM 2-deoxy-D-ribose, 1 mL of the different concentrations of xylitol (1.25, 2.5, 5, and 10% w/v) or positive control (GA) and 400 µL of 50 mM sodium phosphate buffer (pH 7.4) were added into a test tube sequentially and incubated at 50°C for 2 h. Thereafter, 1 mL of 2.8% TCA and 1 mL of 1.0% thiobarbituric acid (in 0.025 M NaOH) were added and the mixture was incubated at 50°C for another 30 min. After incubation, the absorbance was recorded at 532 nm and OH· scavenging activity was calculated using the following formula:

OH· scavenging activity = [1- (Absorbance of sample/Absorbance of control)] × 100

Nitric oxide radical (NO·) scavenging assay

This was carried out according to the method described by Ibrahim et al. (16). A 500 µL of 10 mM sodium nitroprusside in phosphate buffer (pH 7.4) was incubated with 500 µL of the different concentrations of xylitol at 37°C for 2 h. Thereafter, the reaction was started with 500 µL of Griess reagent. Absorbance was measured at 546 nm and NO· scavenging activity of xylitol and positive control (GA) were estimated according to the following formula:

NO· scavenging activity = [1- (Absorbance of sample/Absorbance of control)] x 100

Ferric (Fe³⁺) reducing antioxidant power assay

This was also carried out according to the method as described by Ibrahim et al. (16). Briefly, 1 mL of the increasing concentrations of xylitol (1.25–10% w/v) was incubated with 1 mL of 1% potassium ferricyanide (in 0.2 M sodium phosphate buffer, pH 6.6) at 50°C for 30 min, and thereafter, one mL of 10% TCA was added to the mixture. Reaction was started with 1 mL of distilled water and 200 µL of 0.1% ferric (Fe^{3+}) chloride and the absorbance was measured at 700 nm. The ferric reducing power of xylitol and positive control (AA) was calculatd as per following formula:

Ferric reducing power (%) = (Absorbance of sample/Absorbance of control) x 100

In vivo study
Animals

Twenty-four (24) seven-week-old male Sprague-Dawley rats with mean body weight 192.73

± 22.79 g were procured from the Biomedical Resource Unit located at the Westville Campus of the University of KwaZulu-Natal, Durban, South Africa. They were housed in a temperature- and humidity-controlled room with a 12-h light-dark cycle as two-in-one medium-sized poly carbonated cages. They were acclimatized for one week before the experiment. Animals were divided into 4 groups of 6 animals as follows: Group 1 – normal control (NC); Group 2 – diabetic Control (DBC); Group 3 – normal xylitol (NXYL); Group 4 – diabetic xylitol (DXYL).

Groups 1 and 2 were supplied with normal drinking water, while groups 3 and 4 were supplied with a 10% xylitol solution *ad libitum* instead of drinking water. All animals were fed with a commercial rat pellet diet during the entire experimental period and were maintained according to the rules and regulations of the Experimental Animal Ethics Committee of the University of KwaZulu-Natal, Durban, South Africa.

Induction of type 2 diabetes

A 10% fructose solution was supplied in the form of drinking water for first two weeks to the animals in DBC and DXYL groups (groups 2 and 4) to induce insulin resistance, thereafter, they were injected with a single dose (i.p.) of streptozotocin (65 mg/kg body weight) dissolved in a citrate buffer (pH 4.5) to induce partial pancreatic β-cell dysfunction. Groups 1 and 3 were injected with citrate buffer only. One week after the streptozotocin injection, rats with blood glucose level > 300 mg/dL were considered as diabetic, and used for the study.

Collection of blood and organs

At the end of the 5 weeks intervention period, animals were euthanized by halothane anesthesia and the blood was collected *via* cardiac puncture and refrigerated immediately for further processing. The blood was centrifuged at 3000 rpm for 15 min to obtain serum which was preserved at –30°C for subsequent analysis. Liver, heart, pancreas and kidney samples were collected from each animal, washed with cold normal saline, wiped with filter paper, weighed and preserved at –30°C for further analysis.

Preparation of tissue homogenates

Tissue homogenates were prepared by homogenizing a 0.5 g of tissue samples (liver, heart, kidney and pancreas) in 5 mL of ice cold homogenization buffer (50 mM sodium phosphate buffer with triton X-100, pH 7.5) using liquid nitrogen and ceramic mortar and pestle. Homogenates were then centrifuged at 15000 rpm for 15 min at 4°C (Eppendorf 5424R, Hamburg, Germany). The supernatants were collected and stored at -20°C for further analysis.

Estimation of total protein

Total protein of the tissue homogenates and serum was determined using LabMax Plenno Chemistry Analyzer (Labtest Co. Ltd., Costa Brava, Brazil) and unit was expressed as g/dL.

Determination of in vivo anti-oxidative parameters

Lipid peroxidation was determined by measuring thiobarbituric acid reactive (TBAR) substance as malondialdehyde (MDA) equivalent as described previously by Chenni et al. (17). Catalase (CAT) activity was estimated by measuring the rate of decomposition of H_2O_2 (18). The superoxide dismutase (SOD) activity was determined by the method of Ellerby and Bredesen (19). Glutathione peroxidase (GPx) activity was measured by the method of Flohé and Günzler (20) with slight modifications, while the method described by Ray et al. (21) was adopted in estimating the reduced glutathione (GSH) content in different samples.

Statistical analysis

Data are presented as the mean ± SD of 5-7 animals. All data were analyzed using one-way analysis of variance (ANOVA) and Tukey's HSD *post-hoc* test by using a statistical software package (IBM, SPSS, version 21, NY, USA) and p values < 0.05 were considered significant, when comparing between the groups.

RESULTS

In vitro study
Free radical scavenging activity of xylitol

The results of free radical scavenging activity of the different concentrations of xylitol are presented in Table 1. A significant (p < 0.05) increase in OH· and NO· scavenging activity was observed with increasing concentrations of xylitol. Although NO· scavenging activity was increased dose dependently with the increasing concentrations of xylitol, no significant difference was observed in terms of OH· scavenging activity when higher concentrations (2.5% – 10%) of xylitol were used. The OH· scavenging activities of the higher concentrations of xylitol were significantly (p < 0.05) better than the 50 μg/mL GA, while 10% xylitol showed comparable but significantly lower NO· scavenging activity compared to GA (Table 1).

Table 1. Percentage hydroxyl and nitric oxide radical scavenging activities (relative to 50 µg/mL gallic acid) of different concentrations of xylitol.

	Free radical scavenging activity (%)	
Samples tested	Hydroxyl radical	Nitric oxide radical
1.25% Xylitol	78.71 ± 1.79[a*]	19.27 ± 4.19[a*]
2.5% Xylitol	86.75 ± 1.08[ab*]	22.99 ± 3.22[a*]
5% Xylitol	89.65 ± 0.96[b*]	37.85 ± 3.63[b*]
10% Xylitol	91.83 ± 1.13[b*]	40.63 ± 2.8[b*]
50 µg/mL Gallic acid	69.49 ± 3.24[c]	45.16 ± 0.83[c]

Data are presented as the mean ± SD values of triplicate determinations. [a-c]Different superscript letters presented within a column for a given parameter are significantly different from each other and *significantly different from the positive control (gallic acid) ($p < 0.05$, Tukey's HSD multiple range *post-hoc* test, IBM, SPSS, version 21).
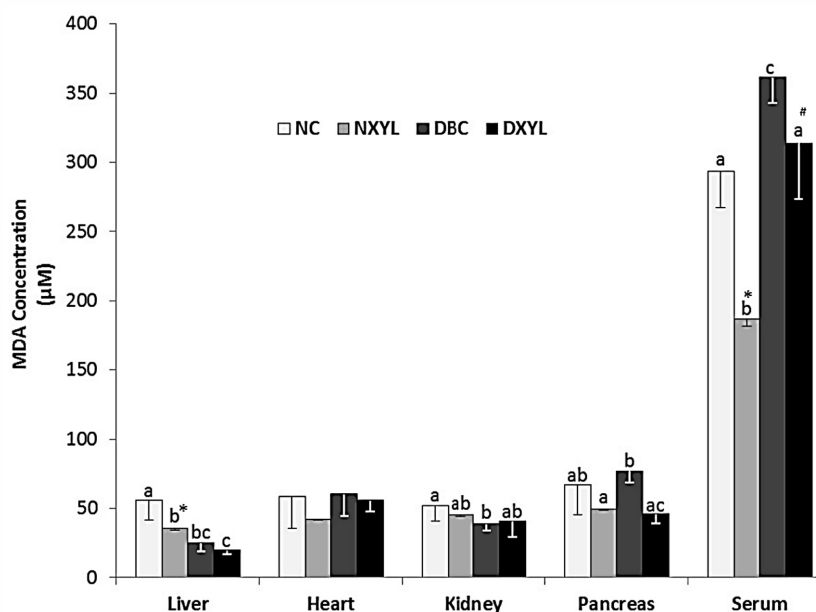

Figure 1. Thiobarbituric acid reactive substances as malondealdehyde (MDA) equivalent in the various samples of different animal groups at the end of experimental period. Data are presented as the mean ± SD of 5 to 6 animals. [a-c]Different letters presented above the bars for a given sample are significantly different from each other group of animal, *significantly different from NC group and #significantly different from DBC group ($p < 0.05$, Tukey's HSD multiple range *post-hoc* test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol

Ferric reducing power of xylitol

The results of ferric reducing power of the different concentrations of xylitol are presented in Table 2. Although increasing ferric (Fe^{3+}) reducing power was observed with increasing concentrations of xylitol, no significant difference was observed between the ferric reducing power of 1.25% and 2.5% as well as 5% and 10% concentrations of xylitol. The ferric reducing power of the different concentrations of xylitol was significantly lower than the 50 µg/mL of AA in this study.

***In vivo* study**

Effect of xylitol on thiobarbuturic acid reactive substances (TBARS)

The results of TBARS as equivalent of MDA are presented in Figure 1. Induction of diabetes led to increase MDA concentration in the serum, cardiac and pancreatic tissues of the experimental groups indicating an onset of oxidative stress. This was significantly ($p < 0.05$) reduced in the serum and pancreas after the treatment with xylitol. However, reduced MDA concentration was

observed in the hepatic and renal tissues of the diabetic group as compared to the normal group when the results were significantly different only for the

kidney sample. Although feeding xylitol did not show any significant effect on kidney tissue, significantly lower MDA concentration was observed in
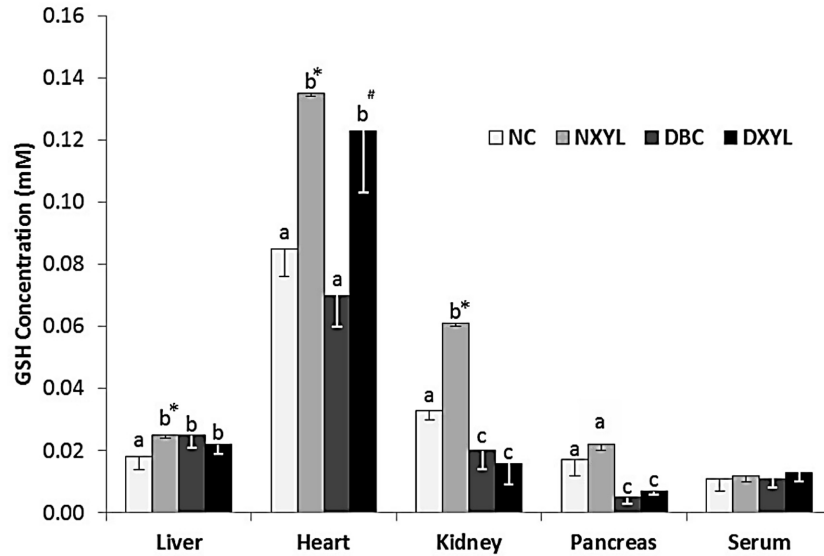

Figure 2. Reduced glutathione (GSH) concentration in the various samples of different animal groups at the end of the intervention period. Data are presented as the mean ± SD of 5 to 6 animals. [a-c]Different letters presented above the bars for a given sample are significantly different from each other group of animals, *significantly different from NC group and [#]significantly different from DBC group ($p < 0.05$, Tukey's HSD multiple range *post-hoc* test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol
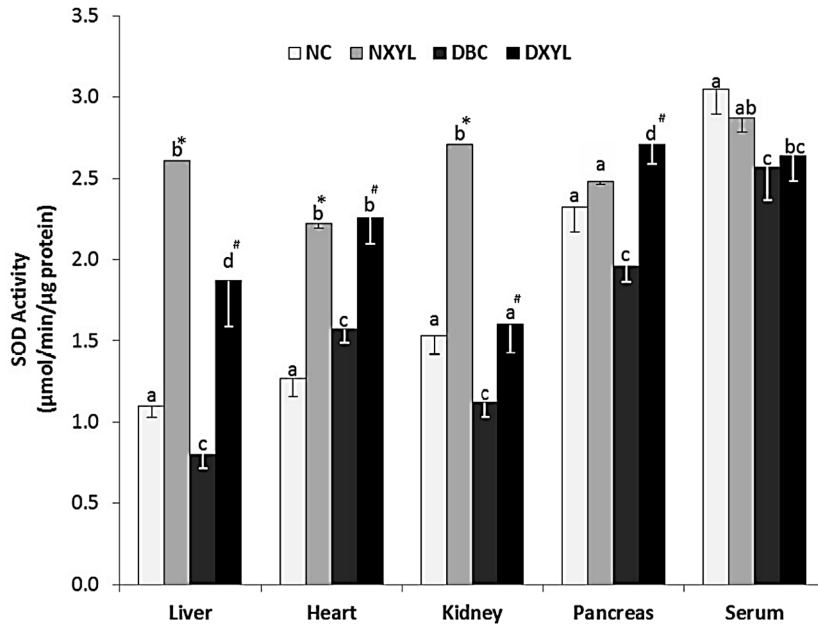

Figure 3. Superoxide dismutase (SOD) activity in the various samples of different animal groups at the end of the intervention period. Data are presented as the mean ± SD of 5 to 6 animals. [a-d]Different letters presented above the bars for a given sample are significantly different from each other group of animals, *significantly different from NC group and [#]significantly different from DBC group ($p < 0.05$, Tukey's HSD multiple range *post-hoc* test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol

the hepatic tissue of xylitol-fed normal and diabetic animals.

Effect of xylitol on reduced glutathione concentration

The results of GSH concentration in the various samples of the different animal groups are shown in Figure 2. A reduced GSH concentration was observed in the all studied tissues of the diabetic group compared to their respective controls except hepatic tissue. However, the results were only significantly different in the cardiac tissue. Feeding xylitol not only reversed the level of GSH in the cardiac tissue of diabetic rats but also significantly increased the levels of GSH in the all other organ tissues (Fig. 2).

Effect of xylitol on superoxide dismutase and catalase activities

The results of SOD and CAT activity are presented in Figures 3 and 4, respectively. The SOD and CAT activities were significantly decreased in the all studied tissues and serum of the diabetic rats as compared to the control group which were significantly ($p < 0.05$) reversed by the feeding of xylitol. Feeding of xylitol also significantly increased the SOD activity in the all studied organs (Fig. 3), while catalase activity was increased in the liver, pancreas and serum of the xylitol consuming normal rats (Fig. 4).

Effect of xylitol on glutathione reductase activity

The results of GR activity are shown in Figure 5. Although the induction of diabetes did not cause any significant ($p < 0.05$) reduction in GR activities in the serum and most tissues, its activity was significantly increased in the liver and serum of diabetic rats (Fig. 5). Xylitol feeding significantly ($p < 0.05$) reduced the induced GR activities in the liver, heart and serum samples compared to the DBC group (Fig. 5). Contrarily, xylitol feeding significantly ($p < 0.05$) increased the GR activity in the pancreas of normal rats.

Effect of xylitol on glutathione peroxidase activity

The results of GPx activity are shown in Figure 6. The induction of diabetes reduced the GPx activities in all organs. On the other hand, xylitol feeding restored the GPx activity when significant induction was observed in the liver tissue of diabetic rats. Similarly, xylitol feeding significantly increased the GPx activity in the liver and pancreas, when opposite results were observed in the kidney of normal rats (Fig. 6).

DISCUSSION

Oxidative stress has been demonstrated to play a major role in the etiology of diabetic complications (22, 23). The present study was conducted to examine the anti-oxidative effects of xylitol not only
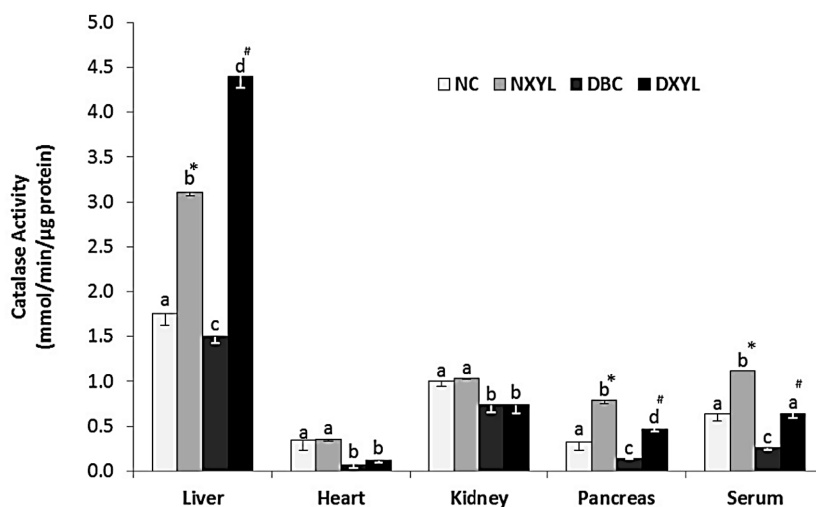
Figure 4. Catalase enzyme activity in the various samples of different animal groups at the end of the intervention period. Data are presented as the mean ± SD of 5 to 6 animals. [a-d]Different letters presented above the bars for a given sample are significantly different from each other group of animals, *significantly different from NC group and #significantly different from DBC group ($p < 0.05$, Tukey's HSD multiple range *post-hoc* test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol
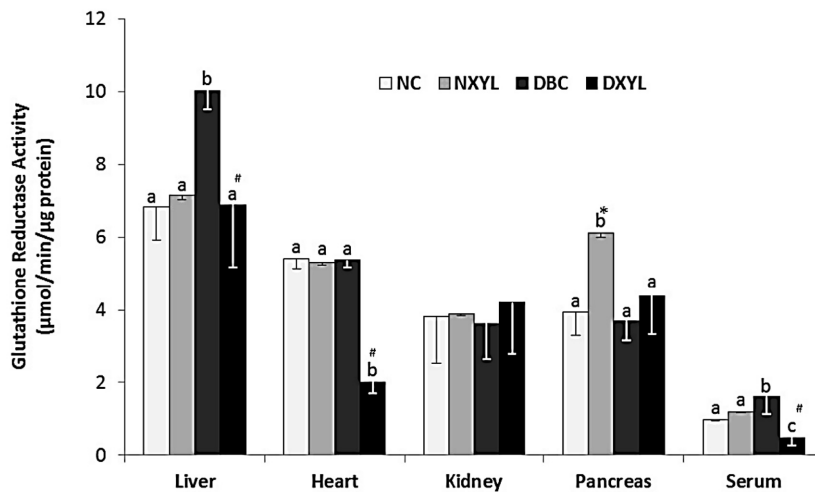
Figure 5. Glutathione reductase activity in the various samples of different animal groups at the end of the intervention period. Data are presented as the mean ± SD of 5 to 6 animals. [a-c]Different letters presented above the bars for a given sample are significantly different from each other group of animals, *significantly different from NC group and #significantly different from DBC group ($p < 0.05$, Tukey's HSD multiple range *post-hoc* test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol
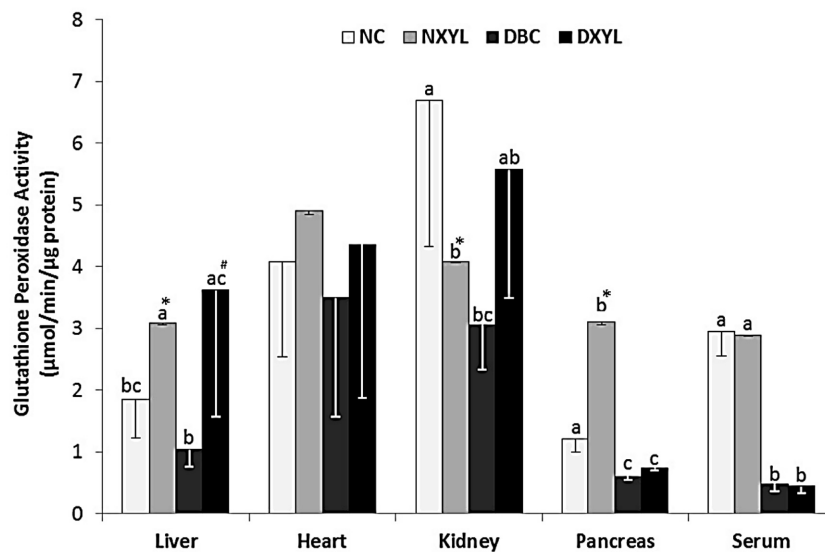

Figure 6. Glutathione peroxidase activity in the various samples of different animal groups at the end of the intervention period. Data are presented as the mean ± SD of 5 to 6 animals. [a-c]Different letters presented above the bars for a given sample are significantly different from each other group of animals, *significantly different from NC group and #significantly different from DBC group ($p < 0.05$, Tukey's HSD multiple range *post-hoc* test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol

in vitro but also in normal and T2D rat model. Different models for *in vitro* anti-oxidative studies were used in our study in order to rationally validate the anti-oxidative potentials of xylitol.

Increased free radicals have been shown to have strong implications on the induction of oxidative stress as well as pancreatic β cell damage and hydroxyl radical (OH·) has been reported as a major

culprit in this regard (24). In our study, the observed OH· and NO· scavenging activity of xylitol indicates its protective effect against free radicals (Table 1). This antioxidant activity of xylitol may be attributed to the free radical scavenging mechanism of polyols as proposed by Faraji and Lindsay (25).

The electron donating and reducing abilities of bioactive compounds have been linked to their

antioxidant activity, which is often demonstrated by their ferric reducing power (16). In our study, the concentration-dependent anti-oxidative effect exhibited by xylitol as ferric reducing power (Table 2) again portrays its potentials as a reducing agent, hydrogen donor and ROS quencher (16).

Additionally, lipid peroxidation has been identified as a major marker of oxidative stress in physiological system which can be determined by measuring the TBARS as MDA equivalent (17). It is a cumulative effect of ROS, which disturbs the assembly of the membrane causing changes in fluidity and permeability, alterations of ion transport and inhibition of metabolic processes (26). In our study, the significantly ($p < 0.05$) reduced concentration TBARS in xylitol consuming normal rats compared to the control again indicates the protective potential of xylitol against oxidative stress (Fig. 1). Furthermore, the significantly reduced concentrations of TBARS in the pancreas and serum of DXYL group compared to the DBC group (Fig. 1) confirms its potential against diabetes-induced lipid peroxidation.

Reduced glutathione (GSH), a physiological marker of oxidative stress, has been reported as one of the most prominent non-enzymatic antioxidants which counteract free-radical mediated damage (1). The lower level of GSH in diabetes mellitus has been linked to the production of hyperglycemia-induced ROS and oxidative stress, which may also reduce the activity of GPx (27). Previous studies have reported that xylitol keeps the glutathione antioxidant system active *via* generating NADPH when metabolized (28, 29) and decreasing oxidized and reduced glutathione ratio (11), which can help to quench free radicals and thereby reduces oxidative damage in the liver as well as in muscle and blood cells. In our study, although the GSH concentrations

of most organs was not affected by the feeding of xylitol in diabetic rats, the reduced concentration of GSH in the other organs of DBC group compared to the NC group indicates the induction of oxidative stress in diabetic condition. On the other hand, the significantly ($p < 0.05$) higher GSH concentration in the all organs of xylitol consuming normal rats and in the heart of xylitol consuming diabetic rats compared to their respective controls again portrays the beneficial effects of xylitol in improving *in vivo* anti-oxidative status in both normal and diabetic condition (Fig. 2).

Superoxide dismutase (SOD) and CAT are two important anti-oxidant enzymes that counteract the harmful effects of free radicals. Superoxide dismutase catalyzes the reduction of superoxide ion to toxic hydrogen peroxide, which is decomposed or reduced to water by CAT and GPx enzymes (30). Recent studies reported that the significantly lower activities of SOD and CAT in the tissues and blood of untreated diabetic rats compared to non-diabetic control, which is similar to findings from our study, was as a result of the induced oxidative stress in diabetic rats (22, 23). However, the significantly ($p < 0.05$) increased activities of SOD and CAT in both xylitol fed normal and diabetic rats compared to their respective controls in our study suggest a potent antioxidant property of xylitol in both normal and diabetic conditions (Figs. 3 and 4).

Glutathione reductase (GR) and GPx are two important glutathione-dependent physiological anti-oxidant enzymes that work in concert to mob up ROS produced in the body. Glutathione peroxidase (GPx) catalyzes hydrogen peroxide and organic hydroperoxides reduction using GSH, while GR catalyzes the NADPH-dependent reduction of oxidized glutathione resulting from GPx catalysis to form GSH (31). Previous studies reported decreased GPx

Table 2. Ferric (Fe^{3+}) reducing power (relative to 50 μg/mL ascorbic acid) of the different concentrations of xylitol.

Samples tested	Ferric reducing power (%)	
1.25% Xylitol	2.97	± 0.93[a*]
2.5% Xylitol	4.32	± 0.93[a*]
5% Xylitol	11.49	± 2.00[b*]
10% Xylitol	15.37	± 1.92[b*]
50 μg/mL Ascorbic acid	64.03	± 2.35[c]

Data are presented as the mean ± SD of triplicate determinations. [a-c]Different superscript letters presented within a column are significantly different from each other and *significantly different from the positive control (ascorbic acid) ($p < 0.05$, Tukey's HSD multiple range *post-hoc* test, IBM, SPSS, version 21).

and GR activities in diabetic subjects and suggested that this may be due to low level of GSH in diabetics (31, 32). In our study, although induction of diabetes did not significantly decrease GR activities (Fig. 5), the significantly ($p < 0.05$) reduced GPx activities and GSH concentration in organs and serum samples of animals in the DBC group compared to the NC group (Figs. 6 and 2) indicates a weaker glutathione-dependent antioxidant defense in diabetic condition. However, the significantly ($p < 0.05$) increased GPx activity in the tissues of diabetic animals after feeding of xylitol (Fig. 6) indicates an improvement in the diabetes-associated depletion of the glutathione-dependent antioxidant defense, and again portrays the beneficial effects of xylitol to improve *in vivo* anti-oxidative status in diabetic condition.

In conclusion, results of this study suggest that xylitol has potential anti-oxidative effects both in normal and type 2 diabetic conditions. This may also be partly involved in pancreatic β cells protecting and glucose lowering effects which have been seen in some in recently published studies (6, 7, 9). Hence, xylitol can be used as a potential supplement in anti-diabetic foods not only as a sweeter or to reduce hyperglycemia but also to ameliorate diabetes associated oxidative stress and other associated metabolic disorders.

Acknowledgment

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Mani D., Shivashankar M.: Int. J. Pharm. Pharm. Sci. 3, 22 (2011).
2. Pourghassem-Gargari B., Abedini S., Babaei H., Aliasgarzadeh A. et al.: J. Med. Plant Res. 5, 2029 (2011).
3. Tangvarasittichai S.: World J. Diabetes 6, 456 (2015).
4. Maritim A.C., Sanders R.A., Watkins J.B.: J. Biochem. Mol. Toxicol. 17, 24 (2003).
5. Mäkinen K.K.: Int. J. Dent. 2010, 981072 (2010).
6. Islam M.S.: J. Med. Food 14, 505 (2011).
7. Islam M.S., M. Indrajit M.: Ann. Nutr. Metab. 61, 57 (2012).
8. Chukwuma C.I., Islam M.S.: Food Funct. 6, 955 (2015).
9. Rahman M.A., Islam M.S.: J. Food Sci. 79, 1436 (2014).
10. Kishore P., Kehlenbrink S., Hu M., Zhang K., Gutierrez-Juarez R. et al.: Diabetologia 55, 1808 (2012).
11. Asakura T., Adachi K., Yoshikawa H.: J. Biochem. 67, 731 (1970).
12. Al-Shahrani M.M., Zaman G.S., Amanullah A.: J. Nutr. Food Sci. 3, 3 (2013).
13. Kang Y., Jo S., Yoo J., Cho J., Kim E. et al.: FASEB J. 24, 829 (2014).
14. Knuuttila M.L., Kuoksa T.H., Svanberg M.J., Mattila P.T., Karjalainen K.M. et al.: Life Sci. 67, 283 (2000).
15. Salmela P.I., Oikarinen A., Pirttiaho H., Knip M., Niemi M. et al.: Diabetes Res. 11, 115 (1989).
16. Ibrahim M.A., Koorbanally N.A., Kiplimo J.J., Islam M.S.: J. Med. Plants Res. 6, 4176 (2012).
17. Chenni A., Yahia D.A., Boukortt F.O., Prost J., Lacaille-Dubois M.A., Bouchenak M.: J. Ethnopharmacol. 109, 207 (2007).
18. Aebi H.: in Methods of Enzymatic Analysis, ed. Bergmeyer H.U., pp. 673-684, Academic Press, New York 1974.
19. Ellerby L., Bredesen D.: Methods Enzymol. 322, 413 (2000).
20. Floché L., Günzler W.A.: Methods Enzymol. 105, 114 (1984).
21. Ray S.D., Chowdhury P., Ray S.: FABAD J. Pharm. Sci. 33, 1 (2008).
22. Sindhu R.K., Koo J.R., Roberts C.K., Vaziri N.D.: Clin. Exp. Hypertens. 26, 43 (2004).
23. Erukainure O.L., Ebuehi O.A.T., Adeboyejo F.O., Aliyu M., Elemo G.N.: Toxicol. Rep. 1, 445 (2014).
24. Gille L., Chott-Ohly P., Fresen N., Schulte I.M., Walde S. et al.: Pharmcol. Toxicol. 90, 317 (2002).
25. Faraji H., Lindsay R.C.: J. Agric. Food Chem. 53, 736 (2005).
26. Nigam S., Schewe T.: Biochim. Biophys. Acta 1488, 167 (2000).

27. Rotruck J., Pope A., Ganther H., Swanson A.: Science.179, 588 (1973).
28. Pösö A.R.: Acta Chem. Scand. B. B33, 93 (1979).
29. Mäkinen K.K.: Med. Hypotheses 54, 603 (2000).
30. Kohen R., Nyska A.: Toxicol. Pathol. 30, 620 (2002).
31. Dincer Y., Alademir Z., Likova H., Akcay T.: Clin. Biochem. 35, 297 (2002).
32. Waggiallah H., Alzohairy M.: N. Am. J. Med. Sci. 3, 344 (2011).