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 surpass 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

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

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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

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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-hydroxydopamine, diethylenetriamine – pentaacetic acid, glutathione reductase (GR), sodium azide, Tris-HCl, L-glutathione oxidized, NADPH, trichloroacetic acid (TCA), Griess reagent, sodium nitroprusside, potassium ferricyanide, 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 FeCl₃ and 100 µM EDTA (1 : 1 v/v) solution, 100 µL of 10 mM H₂O₂, 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:

\[ \text{OH· scavenging activity} = \left[ 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \right] \times 100 \]

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:

\[ \text{NO· scavenging activity} = \left[ 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \right] \times 100 \]

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 (Fe³⁺) chloride and the absorbance was measured at 700 nm. The ferric reducing power of xylitol and positive control (AA) was calculated as per following formula:

\[ \text{Ferric reducing power (}) = \left( \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100 \]

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 cardic 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 H2O2 (18). The superoxide dismutase (SOD) activity was determined by the method of Ellerby and Breiden (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% ñ 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).
The results of ferric reducing power of the different concentrations of xylitol are presented in Table 2. Although increasing ferric (Fe³⁺) 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.

### 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 (Fe³⁺) 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
Xylitol improves antioxidative defense system in serum, liver, heart, kidney. It 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

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**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-cDifferent 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

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**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-dDifferent 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
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...
Xylitol improves antioxidative defense system in serum, liver, heart, kidney...  

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).

<table>
<thead>
<tr>
<th>Samples tested</th>
<th>Ferric reducing power (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25% Xylitol</td>
<td>2.97 ± 0.93^a*</td>
</tr>
<tr>
<td>2.5% Xylitol</td>
<td>4.32 ± 0.93^a*</td>
</tr>
<tr>
<td>5% Xylitol</td>
<td>11.49 ± 2.00^b*</td>
</tr>
<tr>
<td>10% Xylitol</td>
<td>15.37 ± 1.92^b*</td>
</tr>
<tr>
<td>50 µg/mL Ascorbic acid</td>
<td>64.03 ± 2.35^c</td>
</tr>
</tbody>
</table>

Table 2. Ferric (Fe³⁺) reducing power (relative to 50 µg/mL ascorbic acid) of the different concentrations of xylitol.

Data are presented as the mean ± SD of triplicate determinations. ‘’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.

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Conflict of interest

The authors declare that there is no conflict of interest.

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