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1 Original research article (revised version)

2 Effects of xylitol on carbohydrate digesting enzymes activity, 3 intestinal glucose absorption and muscle glucose uptake: A multi- 4 mode study

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1 **ABSTRACT**

2 The present study investigated the possible mechanism(s) behind the effects of xylitol on
3 carbohydrate digesting enzymes activity, muscle glucose uptake and intestinal glucose
4 absorption using *in vitro, ex vivo* and *in vivo* experimental models. The effect of increasing
5 concentrations of xylitol (2.5% - 40% or 164.31 mM – 2628.99 mM) on alpha amylase and alpha
6 glucosidase activity *in vitro* and intestinal glucose absorption and muscle glucose uptake were
7 investigated in *ex vivo* condition. Additionally, the effects of an oral bolus dose of xylitol (1 g/kg
8 BW) on gastric emptying and intestinal glucose absorption and digesta transit in the different
9 segments of the intestinal tract were investigated in normal and type 2 diabetic rats at 1 hour
10 after the dose administration, when phenol red was used as a recovery marker. Xylitol exhibited
11 concentration-dependent inhibition of alpha amylase (IC_{50} = 1364.04 mM) and alpha glucosidase
12 (IC_{50} = 1127.52 mM) activity *in vitro* and small intestinal glucose absorption in *ex vivo*
13 condition. Xylitol also increased dose dependent muscle glucose uptake with and without insulin,
14 although the uptake was not significantly affected by the addition of insulin. Oral single bolus
15 dose of xylitol significantly delayed gastric emptying, inhibited intestinal glucose absorption but
16 increased intestinal digesta transit rate both in normal and diabetic rats compared to their
17 respective controls. The data of this study suggest that xylitol reduces intestinal glucose
18 absorption via inhibiting major carbohydrate digesting enzymes, slowing gastric emptying and
19 fastening intestinal transit rate but increases muscle glucose uptake in normal and type 2 diabetic
20 rats.

21 ***Keywords:***

22 Carbohydrate digesting enzymes, Intestinal glucose absorption, Muscle glucose uptake, Type 2
23 diabetes, Xylitol

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1 **List of abbreviations: (alphabetical)**

2 DBC, diabetic control

3 DXYL, diabetic xylitol

4 GAI, glucose absorption index

5 GIT, gastrointestinal tract

6 NC, normal control

7 NFBG, non-fasting blood glucose

8 NXYL, normal xylitol

9 PR, phenol red

10 T2D, type 2 diabetes

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1. Introduction

2 Starch from carbohydrates is a major dietary source of glucose, which is produced by the
3 gastrointestinal hydrolysis of starch by α-amylase and α-glucosidase enzymes.[1] Then it is
4 absorbed via small intestinal mucosa and influences postprandial blood glucose levels and
5 hyperglycemic condition in diabetics.[1] Thus, limiting the extent of postprandial glucose
6 production as well as absorption can significantly suppress hyperglycemia as well as other
7 complications in diabetics. This is because, persistent hyperglycemia has been reported as a
8 major culprit for diabetes associated complication in all forms of diabetes, with type 2 diabetes
9 (T2D) having the highest prevalence.[2] About 90-95% of the total diabetic patients are suffering
10 from T2D, which has been defined as a heterogeneous metabolic disorder caused by insulin
11 resistance followed by partial pancreatic beta-cell dysfunction as well as hyperglycemia.[2-4]
12 Recently, there has been a growing interest of using nutraceuticals for the management of
13 hyperglycemia as well as T2D,[5] which includes but not limited to medicinal foods, functional
14 foods and sugar alcohols such as xylitol.

15 Xylitol is a 5 carbon sugar alcohol with lower glycemic index (13 vs 65) and calorific value
16 (2.4 vs 4.0 kcal/g) compared to sucrose.[6,7] A number of previous studies reported that xylitol has
17 many other potential beneficial effects such as control and prevention of obesity, diabetes and
18 related metabolic disorders.[8] In a recent study, Islam[6] reported that 3 weeks supplementation of
19 10% dietary xylitol significantly decreased non-fasting blood glucose (NFBG) and serum
20 fructosamine levels; increased serum insulin levels; and improved glucose tolerance ability
21 compared to 10% sucrose in non-diabetic rats.[6] In a more recent study, Islam and Indarjit[9]
22 reported that 5 weeks supplementation of 10% dietary xylitol significantly reduced NFBG and
23 also improved most of the diabetes-related metabolic parameters in a T2D rat model.[9] On the

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1 other hand, Amo *et al.*[8] reported that 8-week supplementation of 1 or 2 g of xylitol per 100 kcal
2 diet significantly decreased visceral fat mass and plasma lipid concentration in high fat diet-fed
3 rats.[8] In another study, Kishore *et al.*[10] reported that xylitol prevents non-esterified fatty acid-
4 induced insulin resistance in non-diabetic rats.[10] In a most recent study, Rahman and Islam[11]
5 confirmed that xylitol has ability to improve the pancreatic islets morphology to improve T2D in
6 rats. All of the above-mentioned studies reported the anti-diabetic potentials of xylitol, however
7 the effects of xylitol on carbohydrate digesting enzymes activity, intestinal glucose absorption
8 and muscle glucose uptake are still not clear.
9 In some previous studies, it has been reported that xylitol consumption significantly reduced
10 food intake in normal humans[12] and diabetic rats[9]. Slower gastric emptying[12,13] and more
11 accelerated intestinal transit[14] were observed in normal human subjected when xylitol was
12 supplied as single oral dose (30 g in 200 ml water) compared to the similar dose of glucose.
13 Hence, xylitol might reduce NFBG levels not only by reducing food intake but also by slowing
14 gastric emptying and accelerating nutrient transit time both in normal and diabetic conditions.
15 Furthermore, the insulinotropic effect of xylitol in diabetic condition[9] may improve circulating
16 glucose uptake, especially in muscle and fat cells to ameliorate hyperglycemia in diabetics. From
17 the above-mentioned studies, it is not clear whether xylitol has any additional effects on the
18 absorption of glucose from the different segments of the intestinal tract and on the muscle
19 glucose uptake at the post absorption period.
20 Hence, the present study was conducted to examine the effects of xylitol on intestinal glucose
21 absorption as well as muscle glucose uptake using three different set of experiments: (1) *in vitro*:
22 effects of xylitol on α-amylase and α-glucosidase activity; (2) *ex-vivo*: effects of xylitol on
23 glucose absorption in isolated rat jejunum and on glucose uptake in isolated psoas muscles of

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1 normoglycemic rats; and (3) *in vivo*: effects of xylitol on glucose absorption and digesta transit
2 in the different segments of gastrointestinal tracts of normal and type 2 diabetic rats.
3

4 ## 2. Materials and methods

5 ### 2.1 Chemicals and reagents

6 The α-amylase, α-glucosidase, streptozotocin, 3,5-dinitrosalicylic acid, di-basic sodium
7 phosphate, paranitrophenyl-α-D-glucopyranoside and citric acid were purchased from Sigma
8 Aldrich, Germany. Mono-basic sodium phosphate, sodium hydroxide, sodium bicarbonate,
9 sodium chloride, potassium chloride, calcium chloride di-hydrate, mono-basic potassium
10 phosphate, magnesium sulphate, sodium hydrogen carbonate, phenol red, sodium citrate and
11 sodium potassium tartrate were purchased from Merck, South Africa. Xylitol (food grade) was
12 kindly supplied by Sweet Nothings, South Africa. Starch, glucose and fructose were purchased
13 from Associated Chemical Enterprise, South Africa, while metformin and Novo rapid insulin
14 were purchased from a local pharmacy store (Pharmed) in Durban, South Africa.
15

16 ### 2.2 In vitro study

17 #### 2.2.1 Measurement of the alpha amylase inhibitory activity

18 The effect of xylitol on alpha amylase activity was determined using a method described
19 previously[15] with slight modifications. Reducing sugars resulting from starch hydrolysis by α-
20 amylase enzyme can reduce yellow 3,5-dinitrosalicylic acid (DNSA) to a reddish-brown 3–
21 amino-5-nitrosalicylic acid, which can be monitored at 540 nm. Briefly, 1 mL of the different
22 concentrations of xylitol (164.31 – 2628.99 mM) or 0.37 mM acarbose or 0.37 mM of quercetin
23 and 1 mL of 4 U/mL α-amylase (in 20 mM sodium phosphate buffer, pH 6.9) was incubated for

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1 30 min at 37 ^{O}C in test tubes, after which 1 mL of 1% starch solution was added in each tube and
2 the mixture was incubated for 1 h at 37 ^{O}C. A 1 mL of DNSA reagent was added in each tube
3 and boiled for 10 min in a boiling water bath and the absorbance was measured at 540 nm after
4 cooling in a spectrophotometer (UV mini-1240, Shimadzu Corporation, Kyoto, Japan).
5 Percentage inhibition was determined according to the following formula:
6
7 % Inhibition = [(Absorbance of control – Absorbance of sample)/Absorbance of control] x 100
8

9 **2.2.2 Measurement of the alpha glucosidase inhibitory activity**

10 The effect of xylitol on alpha glucosidase activity was measured according to a previously
11 described method[16] with slight modifications. Briefly, 0.5 mL of the different concentrations of
12 xylitol (164.31 – 2628.99 mM) or 0.37 mM acarbose or 0.37 mM of quercetin and 1 mL of 1
13 U/mL α-glucosidase in assay buffer (0.1 M sodium phosphate buffer, pH 6.9) was incubated for
14 10 min at 25 ^{O}C in test tubes. Reaction was started with a 0.5 mL of substrate (paranitrophenyl-
15 α-D-glucopyranoside) at 25 ^{O}C and thereafter stopped with 2 mL of 0.2 M sodium bicarbonate
16 after a 5 min incubation period. Absorbance was measured at 405 nm using above-mentioned
17 spectrophotometer and percentage inhibition was determined as the inhibition of release of
18 yellow p-Nitro phenol from substrate by enzymatic action. Percentage inhibition of α-
19 glucosidase was calculated using the following formula:
20
21 % Inhibition = [(Absorbance of control – Absorbance of sample)/Absorbance of control] x 100
22

23 **2.3 Ex-vivo study**

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1 ### 2.3.1 Animals

2 Five adult male Spagrue-Dawley rats with mean body weight 201.12 ± 12.48 g were procured
3 from the Biomedical Resource Center located at the Westville Campus of the University of
4 KwaZulu-Natal, Durban, South Africa. The animals were fasted over-night (12 hours) and
5 euthanized by halothane anesthesia. The abdominal wall was dissected and the whole
6 gastrointestinal tract (GIT) and parts of the psoas muscle were collected and immediately used
7 for glucose absorption and glucose uptake study, respectively. All animal procedures were
8 carried out according to the rules and regulations of the Experimental Animal Ethics Committee
9 of the University of KwaZulu-Natal, Durban, South Africa (Ethical approval number:
10 097/13/Animal).

11

12 ### 2.3.2 Measurement of glucose absorption in isolated rat jejunum

13 The effect of xylitol on glucose absorption by isolated rat intestine was measured by monitoring
14 the reduction of glucose concentration in incubation solution containing isolated rat jejunum and
15 test samples according to a previously published method[17] with slight modifications. Jejunal
16 segments of the collected GIT were cut into small portions of 5 cm and rinsed with 2 mL of
17 Kreb's buffer (118 mM NaCl, 5 mM KCl, 1.328 mM $CaCl_2.2H_2O$, 1.2 mM KH_2PO_4, 1.2mM
18 $MgSO_4$ and 25 mM $NaHCO_3$) through the jejunal lumen using a sterile syringe. Each segment
19 was first inverted to expose the villi, and then incubated in carbon dioxide (CO_2) incubation tube
20 containing 8 mL of Kreb's buffer and 11.1 mM glucose and different concentrations of xylitol
21 (657.25 mM, 1314.50 mM and 2628.99 mM) when glucose with Kreb's buffer was used as a
22 control. A 1 mL sample was collected from each incubation tube before and after a 2 h
23 incubation in a Steri-Cult CO_2 incubator (Labotec, South Africa) at 5% CO_2, 95% Oxygen and

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1 37 ^{O}C condition and glucose concentration was measured using an automated Chemistry
2 Analyzer (Labmax Plenno, Labtest Inc., Costa Brava, Brazil). The intestinal glucose absorption
3 was calculated as the amount of glucose (mg) absorbed per centimeter of rat jejunum using the
4 following formula:
5
6 $$\text{Intestinal glucose absorption} = (GC_1 - GC_2)/\ 5\text{ cm of jejunum}$$
7 Where, "GC_1" and "GC_2" are glucose concentrations (mg/dL) before and after incubation,
8 respectively.
9
10 **2.3.3 Measurement of glucose uptake in isolated rat psoas muscles**
11 The effect of xylitol on glucose uptake in isolated rat psoas muscles was determined according to
12 the method described in a previous study[18] with slight modifications. Briefly, the collected psoas
13 muscle was immediately rinsed with Kreb's buffer and cut into small pieces of equal weight (500
14 mg). Each piece was then incubated in 8 ml of Krebs'buffer, containing 11.1 mM glucose
15 (control) and increasing concentrations of xylitol (657.25 mM, 1314.50 mM and 2628.99 mM)
16 with and without insulin (50 mU/ml). Incubation period was for 1 h in a CO_2 incubator at 5%
17 CO_2, 95% Oxygen and 37 ^{O}C condition. A 1 mg/mL metformin was used as positive control. A 1
18 mL aliquot was collected from each incubation tube before and after the incubation period and
19 the glucose concentration was measured. Muscle glucose uptake was calculated as the amount of
20 glucose (mg) taken up per gram of muscle tissue using the following formula:
21
22 $$\text{Muscle glucose uptake} = (GC_1 - GC_2)/\ 0.5\text{ g of muscle tissue}$$
23

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Where, "GC_1" and "GC_2" are glucose concentrations before and after incubation, respectively.

2.4 In vivo study

2.4.1 Animals

Twenty-four seven-week-old male Sprague-Dawley (SD) rats with mean body weight 222.08 ±14.49 g were procured from the Biomedical Resource Center located at the Westville Campus of the University of KwaZulu-Natal, Durban, South Africa. Animals were randomly divided into four groups, namely normal control (NC), normal xylitol (NXYL), diabetic control (DBC) and diabetic xylitol (DXYL). Each normal animal group had five animals and each diabetic animal group had seven animals. All animals were fed with a commercial rat pellet diet and were maintained according to the rules and regulations of the Experimental Animal Ethics Committee of the University of KwaZulu-Natal, South Africa during the entire experimental period (Ethical approval number: 097/13/Animal).

2.4.2 Induction of diabetes

In order to induced T2D,[19] during the first 2 weeks of the experiment, the animals in the DBC and DXYL groups were supplied with a 10% fructose solution to induce insulin resistance while the animals in the NC and NXYL groups were supplied with normal drinking water. Thereafter, animals in the DBC and DXYL groups were injected (i.p.) with a low dose of streptozotocin (40 mg/kg body weight) dissolved in citrate buffer (pH 4.5) to induce partial pancreatic β-cell dysfunction.[19] Animals in the NC and NXYL groups were injected with similar volume of citrate buffer only. One week after the streptozotocin injection, NFBG levels of all animals were measured using a portable Glucometer (Glucoplus Inc., Saint-Laurent, Quebec, Canada).

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1 Animals with NFBG levels ≥ 300 mg/dl were considered as diabetic and included in the study.[19]
2 Animals with NFBG levels < 300 mg/dl were excluded from the study.
3
4 *2.4.3 Feeding and sampling*
5 One week after the streptozotocin injection and confirmation of diabetes, all animals were fasted
6 overnight (16 hours) with free access to drinking water only. After fasting, the animals in the
7 NXYL and DXYL groups were administered with an oral bolus dose of xylitol (1 g per kg body
8 weight) with glucose (2 g per kg body weight) containing 0.05% (w/v) phenol red (recovery
9 marker). Only glucose with phenol red (PR) was administered to the animals in NC and DBC
10 groups. Animals were then sacrificed using halothane anesthesia exactly 1 h after the dose
11 administration, without any access to food or drinking water. Gastro-intestinal tract (GIT) from
12 each animal was quickly removed, frozen immediately in liquid nitrogen to prevent the
13 movement of the contents, and preserved immediately at -30°C for further analysis.
14
15 *2.4.4 Sample preparation*
16 Each GIT was thawed and divided into eight segments: stomach; 1st, 2nd, 3rd, and 4th quarter of
17 small intestine; cecum; proximal and distal half of the colon. Content weight of each segment
18 was determined by subtracting the weight of the segment without content from the respective
19 weight with content. Contents and tissues were collected and individually homogenized in ice
20 cold normal saline (Ultra Turrax Tube Drive Work Station homogenizer, IKA Laboratory
21 equipment, Staufen, Germany) and centrifuged twice at 15,000 rpm for 30 min (Hettich Mikro
22 200 microcentrifuge, Hettich Lab Technology, Tuttlingen, Germany) according to the method
23 reported previously with slight modifications.[20] Phenol red concentration was determined

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1 spectrophotometrically (Spectrostar Nano, Bmg Labtech, Offenburg, Germany) with bile acid
2 correction in the supernatants of contents and segments according to a previously published
3 method.[21] Briefly, a 30 µL of supernatant or phenol red standard (concentrations 0.0038% -
4 0.00025%) was mixed with 210 µL of 0.1 M dibasic sodium phosphate solution (pH 10.5). The
5 optical density at 420 nm was subtracted from the optical density at 620 nm (for bile acid
6 correction) to get the final optical density. The concentration as well as the amount of recovered
7 phenol red was calculated from the standard curve. Glucose concentration in the intestinal
8 contents was measured using an Automated Chemistry Analyzer (Labmax Plenno, Labtest Inc.,
9 Costa Brava, Brazil) using commercial assay kits.
10
11 *2.4.5 Calculations*
12 Gastric emptying, glucose absorption index (GAI) and digesta transit were calculated using
13 formula described by Islam and Sakaguchi,[22] and expressed in percentage.
14 Gastric emptying, denoting the emptying time of stomach content was calculated using the
15 following formula:
16
17 $$\text{Gastric emptying (\%)} = [(A - B) / A] \times 100.$$
18
19 Where, "A" is the total amount of PR (g) recovered from GIT; and "B" is the total amount of PR
20 (g) recovered from the stomach.
21

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1 GAI denotes the degree of glucose absorption in each GIT segment. It is the percentage amount
2 of the glucose absorbed passing through a given segment of GIT, and was calculated using the
3 following formula:
4
5 $$\text{GAI (\%) in a given segment of the GIT} = (1 - [(a/b) / (c/d)]) \times 100.$$
6
7 Where, "a" is the amount of glucose (g) recovered from that segment; "b" is the amount of PR
8 (g) recovered from the same segment; "c" is the amount of glucose (g) given to corresponding
9 animal; and "d" is the amount of PR (g) given to the corresponding animal.
10
11 Digesta transit in a particular segment of the intestine is the percentage ratio of the amount of
12 content leaving that segment to the amount reaching the same segment. It was calculated using
13 the following formula:
14
15 $$\text{Digesta transit in a given segment (\%)} = (a / b) \times 100.$$
16
17 Where "a" is the amount of PR (g) recovered from that particular segment of the GIT to the
18 distal colon excluding the amount of PR (g) recovered from that particular segment and "b" is
19 the amount of PR (g) recovered from that particular segment of the GIT to the distal colon.
20
21 **3. Results**
22 **3.1 In vitro study**
23 *3.1.1 Effects of xylitol on alpha amylase and alpha glucosidase activity*

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1 The data for *in vitro* α-amylase and α-glucosidase activity are presented both in Fig. 1 and Table
2 1. The data showed increasing dose-dependent effect of xylitol on α-amylase (a) and α-
3 glucosidase (b) activity from 164.31 mM to 2628.99 mM with corresponding IC_{50} values of
4 1364.04 mM and 1127.52 mM respectively (Fig.1 and Table 1). Although no difference of α-
5 amylase activity was observed for 1314.49, 1971.74 and 2628.99 mM xylitol, their inhibitory
6 activities were significantly higher than the other lower dosages (164.31, 328.62, 657.25 mM)
7 used in the study (Fig. 1b). On the other hand, significant and dose-dependent α-glucosidase
8 activity was observed for different dosage with no difference was observed between 1971.74 and
9 2628.99 mM of xylitol (Fig. 1a). According to the alpha amylase and alpha glucosidase
10 inhibitory effects of acarbose and quercetin (Fig. 1), their IC_{50} values were significantly lower
11 than the xylitol (Table 1).
12
13 **3.2 Ex vivo study**
14 *3.2.1 Effects of xylitol on glucose absorption in isolated rat jejunum*
15 Data showing the effects of xylitol on glucose absorption in isolated rat jejunum are presented in
16 Fig. 2. The results showed that the amount of glucose absorbed by isolated rat jejunum in the
17 presence of xylitol was concentration-dependent. This was lowest at 2628.99 mM (1.75 ± 0.46
18 mg/cm jejunum), which was significantly different ($p < 0.05$) from the control and 657.25 mM
19 xylitol (3.27 ± 0.46 and 3.27 ± 0.67 mg/cm jejunum), respectively but they are significantly
20 different from the result for 1314.49 mM xylitol (2.90 ± 0.78 mg/cm jejunum) (Fig. 2).
21
22 *3.2.2 Effects of xylitol on glucose uptake by isolated rat psoas muscle*

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1 The data for the effects of xylitol on glucose uptake with or without insulin in isolated rat psoas
2 muscle are presented in Fig. 3. Induction of glucose uptake was observed with the increasing
3 concentrations of xylitol with or without insulin when only significantly higher ($p < 0.05$)
4 absorption was observed with 2628.49 mM xylitol and metformin compared to the control.
5

6 **3.3 In vivo study**

7 *3.3.1 Effects of xylitol on gastric emptying*

8 The data of gastric emptying are presented in Fig. 4. Induction of diabetes increased gastric
9 emptying, but was significantly reduced ($p < 0.05$) after feeding xylitol. Feeding of xylitol was
10 also reduced gastric emptying in non-diabetic rats.

11

12 *3.3.2 Effects of xylitol on intestinal glucose absorption*

13 The data of intestinal glucose absorption index (GAI) are presented in Fig. 5. The GAI was
14 greatly affected by the administration of xylitol in both normal and diabetic animals. Feeding of
15 xylitol significantly reduced ($p < 0.05$) glucose absorption in the 1st quarter of the small intestine
16 of normal and diabetic rats when significantly and relatively lower glucose absorption were
17 observed in the 2nd and 3rd quarters of small intestine and distal colon of the xylitol fed normal
18 and diabetic rats, respectively compared to their respective controls (Fig. 5). Feeding xylitol did
19 not significantly affect the absorption of glucose from 4th quarter of the small intestine to the
20 proximal colon of the last intestine.
21

22 **4. Discussion**

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1 Xylitol is widely used as a sugar substitute because of its several beneficial effects on health
2 compared to other commonly used sweeteners. The lower caloric value (2.4 kcal vs 4.0 kcal/g),
3 insulinemic response, glycemic index (13 vs 65) but similar sweetness compared to sucrose has
4 made it more popular to people, especially diabetics. In a recent study, it has been reported that
5 xylitol exhibits significant hypoglycemic effects in normal rats[6] and anti-diabetic effects in a
6 T2D rat model[9]. The present study was conducted to investigate possible mechanisms behind the
7 anti-diabetic effects of xylitol using several *in vitro, ex vivo* and *in vivo* models.

8 The action of carbohydrate hydrolyzing enzymes has a significant effect on postprandial
9 blood glucose level, and delaying the digestion of carbohydrate like starch and sucrose will
10 translate into lower postprandial blood glucose.[1,23] The α-amylase and α-glucosidase inhibitors
11 have gained much popularity as a class of hypoglycemic agents that reduces postprandial blood
12 glucose levels via the above-mentioned mechanism.[24-26] Results from the present study showed a
13 significant *in vitro* inhibition of α-amylase and α-glucosidase enzyme activities by xylitol
14 (Fig.1), which corresponds to the results from a study conducted recently[27] and also indicate that
15 xylitol may possess significant inhibitory effects on carbohydrate digestion. This can translate
16 into lower level of postprandial blood glucose, and may be partly involved in the mechanism
17 behind the reported anti-diabetic effects of xylitol.

18 Most of the glucose we consumed is absorbed from the small intestinal mucosa, however the
19 absorption capacity of the different small intestinal segments is not the same. Although a
20 previous *in vitro* study suggested the high capacity of glucose absorption across all segments of
21 the small intestine,[28] another study confirmed that the mid-small intestine (part of the duodenum
22 and jejunum) having the highest glucose absorption capacity.[29] In the present study, a 2 hour
23 incubation of isolated rat jejunum in a glucose solution showed glucose absorption as high as

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1 3.27 ± 0.46 mg/cm of jejunum without xylitol (Fig. 2). However, in the presence of 2628.99 mM
2 xylitol, there was a significant reduction in the glucose absorption capacity (1.75 ± 0.46 mg/cm
3 of jejunum), which revealed the possible inhibitory potentials of xylitol on intestinal glucose
4 absorption.

5 Additionally, several *in vivo* studies have reported the different absorption patterns of
6 glucose from the small intestine. Bogner *et al*.[30] reported that glucose absorption was highest in
7 the mid-intestine of female chicks, when Lavin[28] suggested the ileum as a highest glucose
8 absorption site in the small intestine of the same species. In another study, Riesenfeld *et al*.[31]
9 confirmed the reduction of glucose absorption with the increasing distance from the pylorous of
10 chicken. They also explained that the difference in glucose absorption capacity in the different
11 segments of the small intestine may be due to of the varied concentrations of glucose. The
12 pattern of glucose absorption in our study (Fig. 5) is also consistent with the findings reported by
13 Pearson *et al*.[28]. However, the significantly reduced ($p < 0.05$) GAI in the xylitol fed groups
14 compared to the controls across the small intestinal segments (more pronounced in the 1st and 2nd
15 quarters) correspond to the results of our ex vivo glucose absorption study (Fig. 2). It also
16 supports the inhibitory potentials of xylitol on intestinal glucose absorption in our study.

17 The rate of nutrient gastric emptying and digesta transit are important factors in gastro
18 intestinal nutrient digestion and absorption.[9] It has also been reported that faster intestinal transit
19 and delayed gastric emptying might be the cause of slower intestinal nutrient absorption and
20 reduced food intake.[12,13] In the present study, induction of diabetes appreciably increased the rate
21 of nutrient gastric emptying (Fig. 4). Although negative energy balance resulting from constant
22 hyperglycemia is known as a major reason for the frequent hunger (polyphagia) and increased
23 food intake often observed as a classical symptom of diabetes, when faster gastric emptying may

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1 also contribute to this effect. Previous studies have demonstrated a correlation between gastric
2 emptying rate and eating behavior.[32,33] In our study, an oral bolus dose of xylitol delayed gastric
3 emptying (Fig. 4) and increased digesta transit rate (Table 1) after 1 h of administration in both
4 normal and diabetic rats, which corresponds to studies previously reported in normal human
5 subjects and experimental animals[12-14] and may also contribute to lower GAI in the proximal half
6 of the small intestine.

7 Among other functions, insulin produced in the body helps to stimulate the uptake of
8 circulating glucose by active respiratory cells for energy production, thus maintaining blood
9 glucose homeostasis.[34] Muller-Hess *et al*.[35] reported that blood glucose and serum insulin was
10 significantly increased by oral administration of 30 or 50 g of xylitol in normal subjects.[35] Other
11 studies reported that 3 to 5 weeks oral administration of 10% (657.25 mM) xylitol increased
12 serum insulin in both normal[6] and diabetic rats[9,11]. Since inadequate circulating glucose uptake
13 could partly contribute to the observed hyperglycemia in diabetic rats,[36] it was therefore rationale
14 to investigate the effects of xylitol on glucose uptake in isolated rat psoas muscle. Results from
15 *ex vivo* investigation in the present study suggest significant potentials of xylitol to improve
16 muscle glucose uptake with or without insulin (Fig. 3), which might partly contribute to the anti-
17 diabetic as well as hypoglycemic potential of xylitol.

18 In summary, results from the *in vitro, ex vivo* and *in vivo* sections of the present study
19 suggest that xylitol possesses potential inhibitory effects on the activities of α-glucosidase and α-
20 glucosidase *in vitro*, and intestinal glucose absorption, especially in the duodenal and jejunal
21 segments *ex vivo* and *in vivo* conditions. Our results also suggest that xylitol prolongs gastric
22 emptying and increases intestinal nutrient transit rate *in vivo* in both normal and diabetic
23 conditions, which may partly contribute to its inhibitory potential on intestinal glucose

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absorption. Furthermore, *in vitro* muscle glucose uptake assay of this study suggest that xylitol may also promote the uptake of circulating glucose by muscle tissue.

In conclusion, data of this study suggest that xylitol exhibits its potential hypoglycemic as well as anti-diabetic effects not only by decreasing the activities of carbohydrate digesting enzymes and intestinal glucose absorption but also by delaying gastric emptying, increasing intestinal digesta transit and muscle glucose uptake. Further clinical study can only confirm the similar effects of xylitol in humans.

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Conflict of interest

There is no conflict of interest within this article.

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1 **TABLE CAPTIONS**

2

3 **Table 1**

4 IC_{50} values for the inhibition of alpha glucosidase and alpha amylase enzymes by xylitol,

5 acarbose and quercetin.

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7 **Table 2**

8 Percent digesta transit in the different segments of the GIT during 1 hour experimental period.

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1 **FIGURE CAPTIONS**

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Figure 1 **Fig. 1** Effect of xylitol and standards (acarbose and quercetin) on alpha glucosidase **(a)** and alpha amylase **(b)** activities in vitro. Data are presented as mean ± SD of triplicates of analysis. [a-e]Different letters presented beside the bars for a given enzyme are significantly different from each other ($p < 0.05$. Tukey's HSD *post-hoc* test, IBM, SPSS, version 21).

Figure 2 Effect of xylitol on glucose absorption in isolated rat jejunum. Data are presented as mean ± SD of five replicates of analysis. [ab]Different letters presented above the bars are significantly different from each other ($p < 0.05$. Tukey's HSD *post-hoc* test, IBM, SPSS, version 21).

Figure 3 Effect of xlitol on glucose uptake with or without insulin in isolated rat psoas muscle. Data are presented as mean ± SD of triplicates of analysis. [ab or xy]Different letters presented above the bars for with or without insulin are significantly different from each other ($p < 0.05$. Tukey's HSD *post-hoc* test, IBM, SPSS, version 21).

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Figure 4 Effects of xylitol on gastric emptying in different animal groups at the end of 1 hour experimental period. Data are presented as mean ± SD of five to six animals. [ab]Different letters presented above the bars are significantly different from each other group of animals ($p < 0.05$. Tukey's HSD *post-hoc* test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol.

Figure 5 Glucose absorption index (GAI) in the different GIT segments of different animal groups at the end of 1 hour experimental period. Data are presented as mean ± SD of five to six animals. [abc]Different letters presented above the bars for a given segment are significantly different from each other group of animals ($p < 0.05$. Tukey's HSD *post-hoc* test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol.

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2 **TABLES**

3

4 **Table 1** IC_{50} values for the inhibition of alpha glucosidase and alpha amylase enzymes by

5 xylitol, acarbose and quercetin.

Sample/ standard	Alpha glucosidase inhibition	Alpha amylase inhibition
	IC_{50} values (mM)	
Xylitol	1127.52 ± 85.93[a]	1364.04 ± 171.12[a]
Acarbose	0.15 ± 0.01[b]	0.32 ±0.03[b]
Quercetin	0.04 ± 0.01[b]	0.23 ± 0.02[b]

6 Data are presented as mean ± SD of triplicates of analysis. . [a,b]Different superscript letters within

7 a column for a given enzyme are significantly different from each other ($p < 0.05$. Tukey's HSD

8 *post-hoc* test, IBM, SPSS, version 21).

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2 **Table 2** Percent digesta transit in the different segments of the intestinal tract during 1 hour

3 experimental period.

GROUP	1st qtr	2nd qtr	3rd qtr	4th qtr	Cecum	Prox. Colon
	Small intestine					**Large intestine**
	Digesta Transit (%)					
NC	93.38 ± 1.77	83.23 ± 3.32	71.25 ± 5.28[a]	65.56 ±5.82	51.71 ± 3.73[a]	48.53 ± 8.09[a]
NXYL	94.53 ± 2.54	86.94 ± 2.20	81.06 ± 2.03[b]	69.20 ± 4.72	38.98 ± 8.13[b]	49.40 ± 6.20[a]
DBC	91.11 ± 1.63	82.65 ± 5.49	70.98 ± 4.83[a]	71.68±14.15	48.61 ±6.17[ab]	58.61 ± 8.10[ab]
DXYL	91.54 ± 0.17	84.24 ± 4.14	81.16 ± 4.42[b]	75.02 ± 4.12	41.31 ± 3.75[b]	54.40 ± 4.55[ab]

4 Data are presented as mean ± SD of five to six animals. [ab]Different letters presented in each

5 column for a given segment are significantly different from each other group of animals ($p <$

6 0.05. Tukey's HSD *post-hoc* test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal

7 Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol.

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2 **FIGURES**

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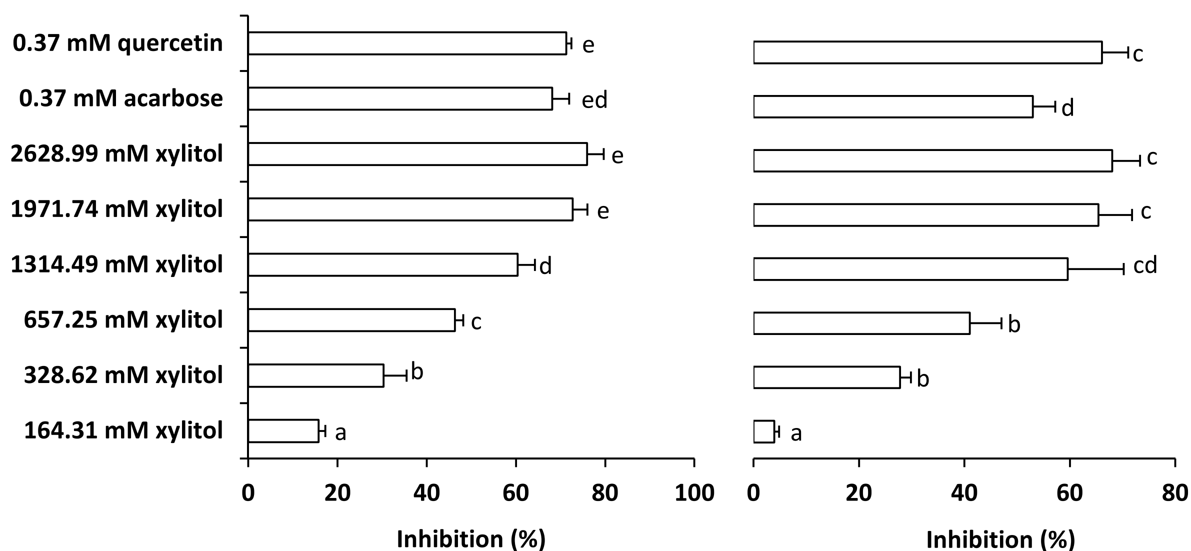

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5 **(a)** **(b)**

6 **Fig. 1** Effect of xylitol and standards (acarbose and quercetin) on alpha glucosidase **(a)** and alpha
7 amylase **(b)** activities in vitro. Data are presented as mean ± SD of triplicates of analysis. [a-
8 e]Different letters presented beside the bars for a given enzyme are significantly different from
9 each other ($p < 0.05$. Tukey's HSD *post-hoc* test, IBM, SPSS, version 21).

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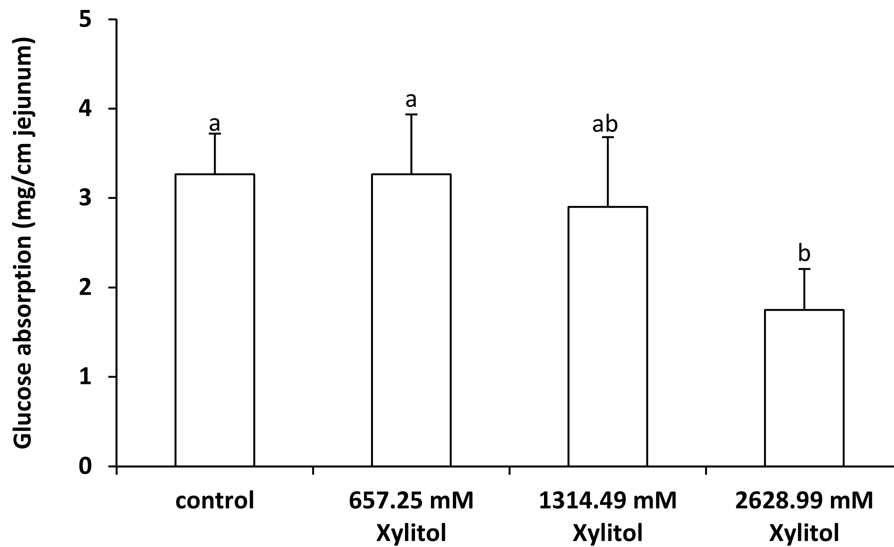

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3 **Fig. 2** Effects of xylitol on glucose absorption in isolated rat jejunum. Data are presented as
4 mean ± SD of five replicates of analysis. [ab]Different letters presented above the bars are
5 significantly different from each other ($p < 0.05$. Tukey's HSD *post-hoc* test, IBM, SPSS,
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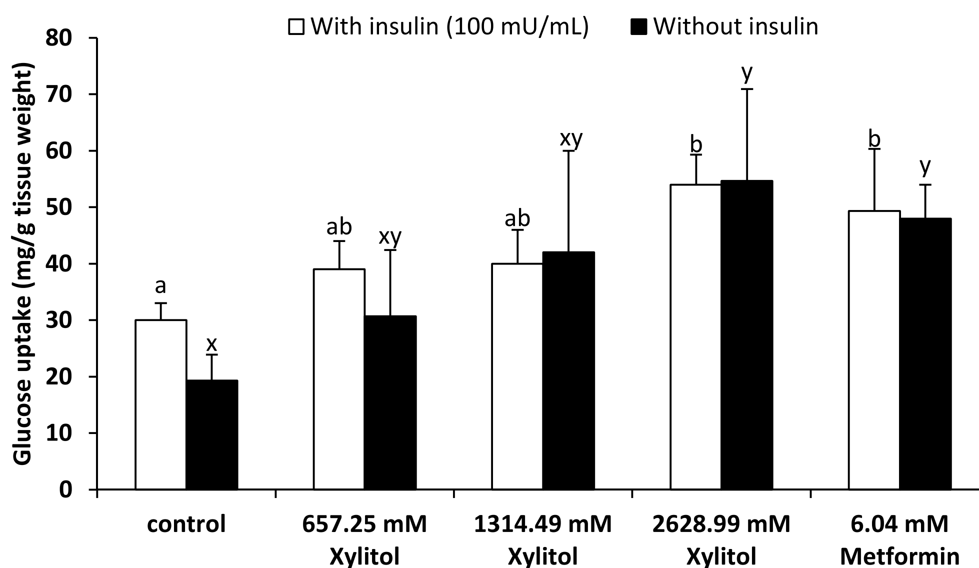

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3 **Fig. 3** Effects of xylitol on glucose uptake with or without insulin in isolated rat psoas muscle.
4 Data are presented as mean ± SD of triplicates of analysis. [ab or xy]Different letters presented above
5 the bars for with or without insulin are significantly different from each other ($p < 0.05$. Tukey's
6 HSD *post-hoc* test, IBM, SPSS, version 21).

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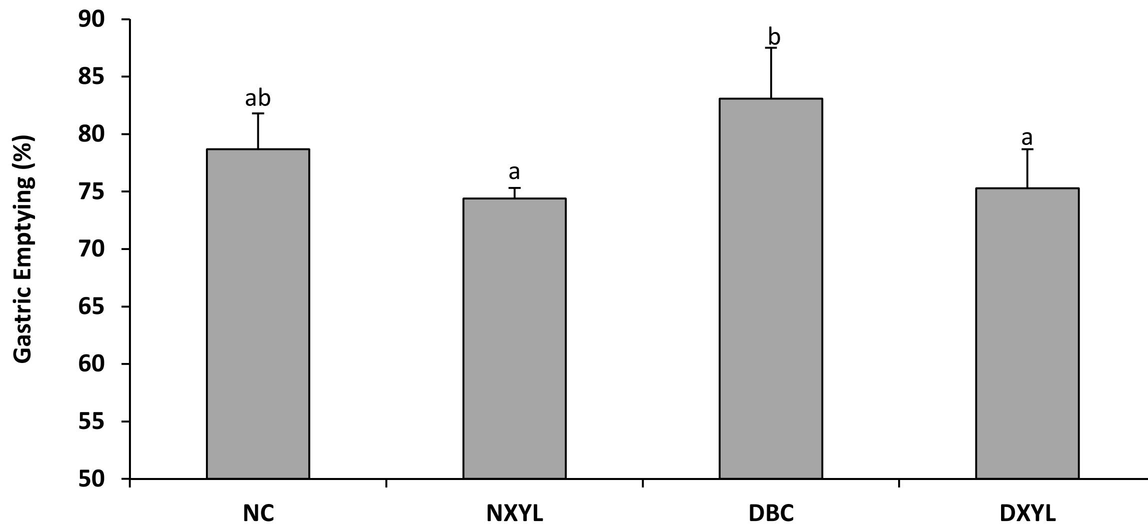

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4 **Fig. 4** Effects of xylitol on gastric emptying in different animal groups at the end of 1 hour
5 experimental period. Data are presented as mean ± SD of five to six animals. [ab]Different letters
6 presented above the bars are significantly different from each other group of animals ($p < 0.05$.
7 Tukey's HSD *post-hoc* test, IBM, SPSS, version 21). NC, Normal Control; NXYL, Normal
8 Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol.

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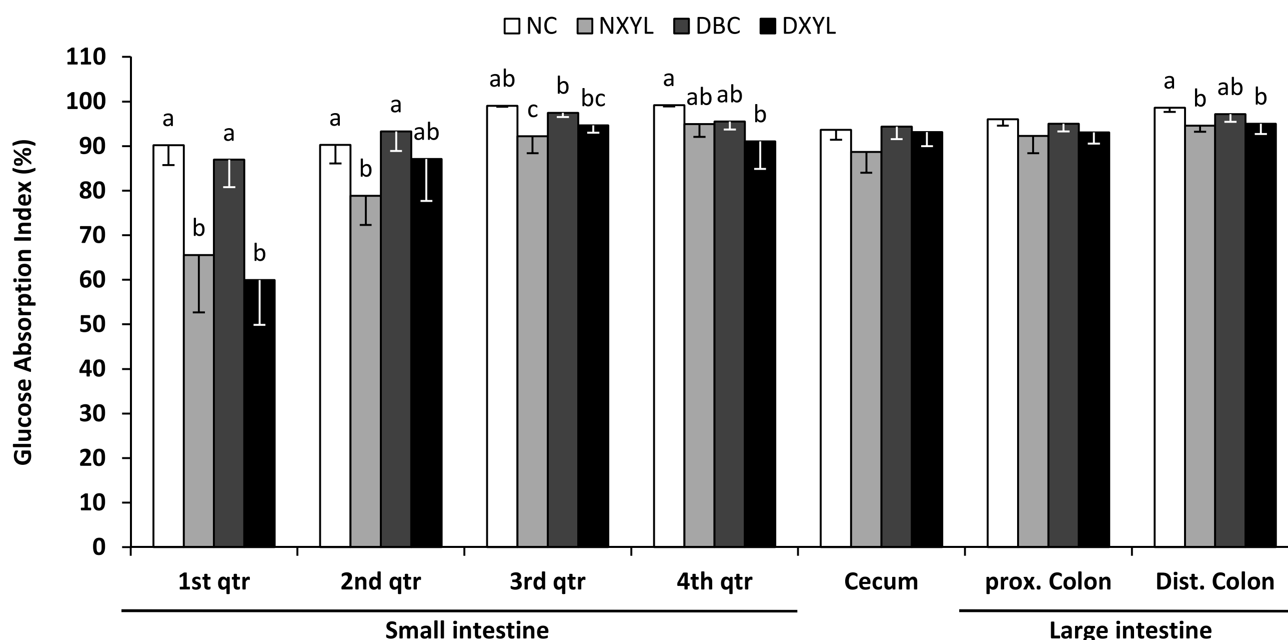

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4 **Fig. 5** Glucose absorption index (GAI) in the different GIT segments of different animal groups

5 at the end of 1 hour experimental period. Data are presented as mean ± SD of five to six animals.

6 [abc]Different letters presented above the bars for a given segment are significantly different from

7 each other group of animals ($p < 0.05$. Tukey's HSD *post-hoc* test, IBM, SPSS, version 21). NC,

8 Normal Control; NXYL, Normal Xylitol; DBC, Diabetic Control; DXYL, Diabetic Xylitol.

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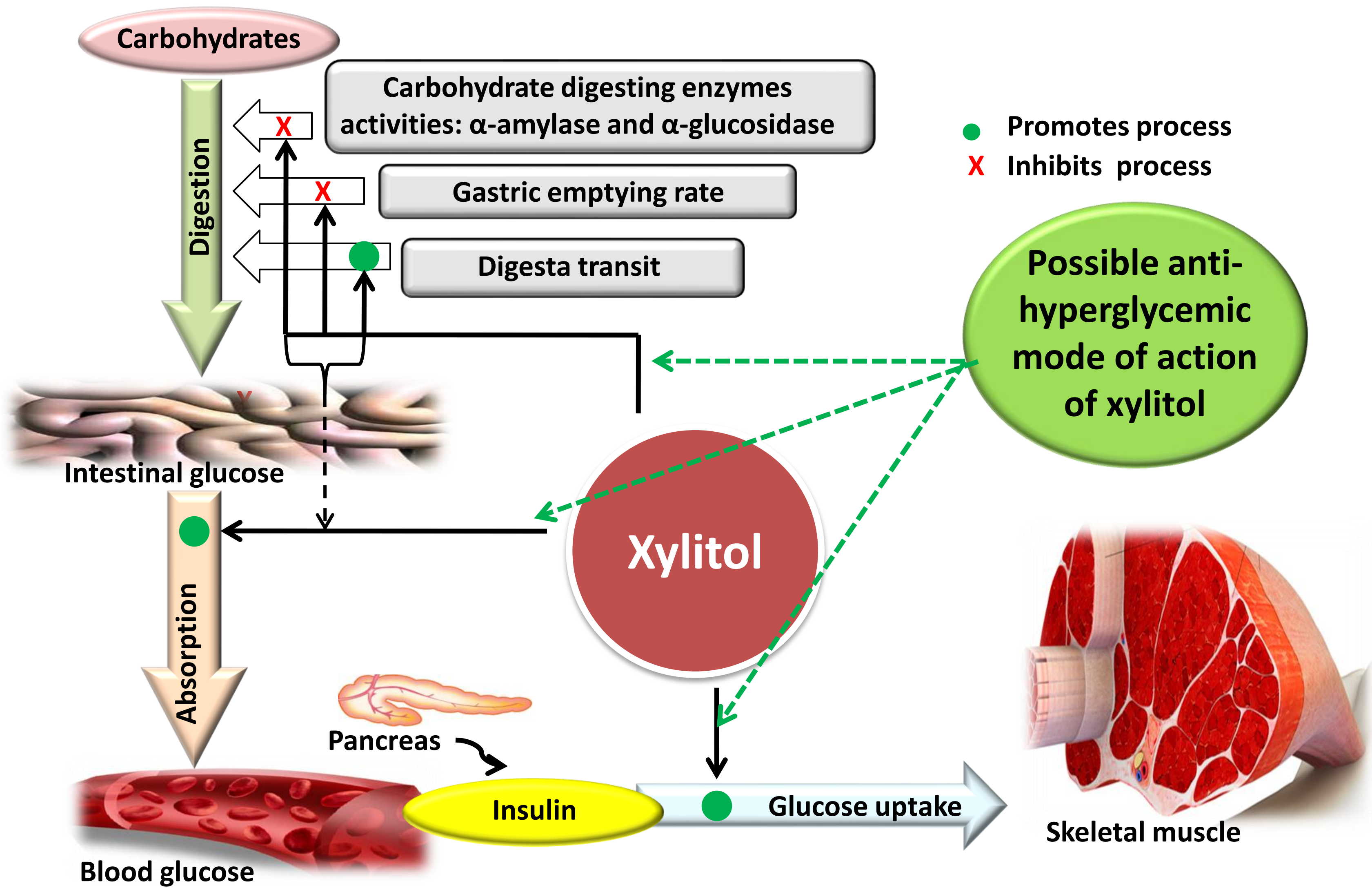
Graphical abstract



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