Studies on the antioxidative and antidiabetic effects of Xylitol and Erythritol in type 2 diabetes: A comparative study

BY

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Student number: 215075542

Submitted in fulfilment of the academic requirements for the degree of Doctor of Philosophy in Biochemistry, School of Life Sciences, University of KwaZulu-Natal (Westville campus), Durban 4000, South Africa

Supervisor: Prof. M.S Islam, PhD

PREFACE

The information presented in this thesis is an original work by the candidate. It was carried out in the Department of Biochemistry, School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Westville Campus, Durban, South Africa from July 2018 to August 2021 under the supervision of Prof. MS Islam and has not otherwise been submitted in any form for any degree or diploma to any other University. Where use has been made of the work of others, it has been duly acknowledged in the text in the form of reference.

Candidate: Nontokozo Zimbili Msomi

Supervisor: Prof. M.S Islam

DECLARATION 1 – PLAGIARISM

I, **Nontokozo Zimbili Msomi**, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. This thesis has not been submitted for any degree or examination at any other university.
3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.
4. This thesis does not contain other persons' writing, unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, then:
    a. Their words have been rewritten but the general information attributed to them has been referenced
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5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed

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Declaration Plagiarism 22/05/08 FHDR Approved

DECLARATION 2 – PUBLICATIONS

Details of contribution to publications that form part and/or include research presented in this thesis (include publications in preparation, submitted, in press and published and give details of the contributions of each author to the experimental work and writing of each publication).

Nontokozo Zimbili Msomi performed all the experiments in the publications and presentations stated below under the supervision of Prof. M.S Islam (PhD), while the co-authors contributed partly to the work. The final editorial work was done by Prof. M.S Islam before submission to the journals for publication.

PUBLICATIONS FROM THIS THESIS

1. **Msomi NZ**, Erukainure OL, Islam MS. Suitability of Sugar Alcohols as Antidiabetic Supplements: A Review. **Journal of Food and Drug Analysis**. 2021;29(1): 1:14

OTHER PUBLICATIONS

2. Erukainure OL, **Msomi NZ**, Beseni BK, Salau VF, Ijomone OM, Koorbanally NA, et al. Cola nitida infusion modulates cardiometabolic activities linked to cardiomyopathy in diabetic rats. **Food and Chemical Toxicology.** 2021,154:112335.
3. Mohamed AI, Beseni BK, **Msomi NZ**, Salau VF, Erukainure OL, Islam MS. The antioxidant and antidiabetic potentials of polyphenolic-rich extracts of Cyperus rotundus (Linn.). (In press, accepted on 8 August 2021, **Journal of Biomolecular Structure and Dynamics**)

SUBMITTED ARTICLES (UNDER REVIEW) FROM THIS THESIS

4. **Msomi NZ**, Islam MS. Xylitol demonstrates better antioxidant and antidiabetic potentials compared to erythritol: A dual approach comparative study. (**Journal of Food Drug Analysis-D-21-00250**)
5. **Msomi NZ**, Erukainure OL, Salau VF, Olofinsan KA, Islam MS. Xylitol improves antioxidant, purinergic and cholinergic dysfunction, and lipid metabolic homeostasis in hepatic injury in type 2 diabetic rats. (**Journal of Food Biochemistry- 08-21-1307)**

PROSPECTIVE PUBLICATIONS FROM THIS THESIS

6. **Msomi NZ**, Islam MS. Comparative effects of xylitol and erythritol on modulating blood glucose; inducing insulin secretion; reducing dyslipidemia and redox imbalance in a type 2 diabetes rat model. (**In preparation**)
7. **Msomi NZ**, Salau VF, Islam MS. Comparative effects of xylitol and erythritol on redox imbalance, cholinergic dysfunction, purinergic activity, and glucose-lipid metabolic homeostasis in psoas muscles of diabetic rats. (**In preparation**)
8. **Msomi NZ**, Salau VF, Beseni BK, Mohamed AI, Olofinsan KA, Islam MS. Comparative effects of xylitol and erythritol on gastric emptying, digesta transit, intestinal glucose absorption and blood glucose in type 2 diabetic rats. (**In preparation**)

PRESENTATIONS

9. **Msomi NZ**, Islam MS. Antioxidant and antidiabetic effects of xylitol and erythritol: A comparative study. **54th Society for Endocrinology, Metabolism and Diabetes of South Africa** – March 2021

10. **Msomi NZ**, Islam MS. *In vitro* and *ex vivo* antioxidant capacity and antidiabetic effect of xylitol and erythritol: A comparative study. **College of Agriculture, Engineering, and Sciences Postgraduate Research & Innovation Symposium**, University of KwaZulu-Natal, Durban, South Africa, December 2020
11. **Msomi NZ**, Islam MS. Antioxidant and antidiabetic effects of xylitol and erythritol: A comparative study. **College of Agriculture, Engineering, and Sciences Postgraduate Research & Innovation Symposium**, University of KwaZulu-Natal, Durban, South Africa, October 2019

DEDICATION

Dedicated to my grandmother Ntombiyenkosi Mirriam Msomi, nomndayi, singila, hlombe, phingoshe, nina enehla ngomzungulu wasala wabola, mangamahle.

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Lastly, my sincere gratitude goes out to my grandmother and my family, thank you for the prayers, love, and support throughout my academic journey.

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LIST OF ABBREVIATIONS

Ace-K	Acesulfame-potassium
AGEs	Advanced glycation end products
ALT	Alanine transaminase
AST	Aspartate transaminase
DAG	Diacylyglycerol
DFD	Diabetic foot disease
DM	Diabetes mellitus
DPP-4	Dipeptidyl-peptidase 4
DPPH	1,1'-diphenyl-2-picrylhydrazyl
DR	Diabetic retinopathy
FRAP	Ferric reducing antioxidant power
GDM	Gestational diabetes mellitus
GIP	Glucose-dependent insulinotropic peptide
GIT	Gastrointestinal tract
GLP-1	Glucagon-like peptide-1
GLUT 4	Glucose transporter 4
GPx	Glutathione peroxidase
GR	Glutathione reductase

GSH	Glutathione
HFCS	High-fructose corn syrup
IDDM	Insulin Dependent Diabetes Mellitus
IDF	International Diabetes Federation
MDA	Malondialdehyde
MODY	Maturity-onset diabetes of the young
NFBG	Non-fasting blood glucose
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NNS	Non-nutritive sweeteners
NO^-	Nitric oxide
OGTT	Oral glucose tolerance test
PKC	Protein kinase C
PR	Phenol red
SGLT2	Sodium-glucose co-transporter
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substance
T1D	Type 1 diabetes
T2D	Type 2 diabetes
WHO	World health Organization

ABSTRACT

The present study comparatively investigated the antioxidative and antidiabetic effects of xylitol and erythritol using *in vitro*, *ex vivo* and *in vivo* experimental models. The free radical scavenging activities (DPPH, nitric oxide (NO) and ferric reducing power (FRAP) and inhibition of carbohydrate digesting enzymes of the increasing concentrations of xylitol and erythritol (90-720 mM) were determined *in vitro*. In *ex vivo* condition, xylitol and erythritol (360-2880 mM) were established for their effects on intestinal glucose absorption and muscle glucose uptake. Additionally, lipid peroxidation (LPO) and reduced glutathione (GSH) concentrations, and the activity of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) enzymes were investigated in Fe^{2+} induced oxidative muscular tissue. Results from these experiments revealed that xylitol exhibited the best activities in terms of all the tested parameters.

Xylitol and erythritol were further investigated for their antidiabetic and antioxidative effects in an intervention trial, using a fructose-streptozotocin induced type 2 diabetes rat model. The trial revealed the effects of these sugar alcohols at different concentrations (5%, 10% and 20%), on food and fluid intake, body weight modification, reducing blood glucose level and enhanced glucose tolerance capability. Upon euthanasia of the animals the curative capability on the complications associated with diabetes was determined on serum lipid profile, insulin resistance, pancreatic β-cell function, liver and muscle function. Oxidative stress biomarkers such as LPO, GSH, SOD and CAT were examined in the serum, pancreas, liver and muscle tissues. The *in vivo* experiment revealed that 10% xylitol dose displayed the most potent antioxidant and antidiabetic properties.

Furthermore, data from the *in vivo* glucose absorption study revealed that xylitol (1 g/kg bw) exhibits better hypoglycemic and antidiabetic effects by delaying gastric emptying,

increasing intestinal transit time and reducing small intestinal glucose absorption compared to erythritol (1 g/kg bw). The overall data of this study suggest that xylitol has a better potency in terms of antioxidative and antidiabetic activities and can be considered as a superior antidiabetic natural sweetening agent over erythritol. However, further clinical studies are required in order to ascertain the results of this study.

CHAPTER 1

Introduction and literature review

1.1 Introduction

Diabetes is a term denoted from the Greek word siphon, as the affected individuals experience polyuria (pass water like a siphon) (Ahmed, 2002; Afolayan and Sunmonu, 2010). Diabetes is classified as mellitus or less often, insipidus (Allan and Rowntree, 1931; Lindholm, 2004). However, the term is often used as diabetes mellitus (Afolayan and Sunmonu, 2010). Diabetes mellitus (DM) is a metabolic disorder characterised by chronic raised plasma glucose levels as a result of inadequate insulin emission, insulin action or both (Asmat *et al*., 2016; Awuchi, Echeta and Igwe, 2020). This is a result of the dysfunction of the pancreatic β-cell, whereby insulin production is impaired or lost (Awuchi *et al*., 2020).

DM is a major public health problem which has reached epidemic proportions, and its prevalence is increasing globally (Al-Lawati, 2017; Zimmet, 2017). In 2000, the World health Organization (WHO) reported that 151 million people were diagnosed with diabetes, corresponding to 4.6% of the global population. This prevalence increased to 9.3% in 2019, with approximately 463 million people living with diabetes worldwide (IDF, 2019b). It was projected in 2016 that DM was the seventh prevalent cause of mortality among diseases like ischaemic heart disease, stroke, chronic obstructive pulmonary disease, lung cancer and dementia (WHO, 2018). It was estimated to have caused 1.6 million deaths globally (WHO, 2020), this figure continues to increase as the number of people with diabetes prolongs to upsurge considerably (IDF, 2019b). This increasing number is due to aging, urbanization, population growth and the escalating prevalence of physical inactivity, sedentary life style,

over consumption of imbalanced and unhealthy foods followed by overweight and obesity (Johnston *et al*., 2013).

Prevention and treatment of diabetes has a major goal of maintaining normal control of glucose metabolism and glycemia (Awuchi *et al.*, 2020). Dietary modification of sugar consumption has been one of the most recommended treatment modality for diabetic patients (Malik and Hu, 2012; Johnston *et al*., 2013). Numerous studies have reported on the consumption of sugar sweetened beverages as a probable link to contributing in an increase in obesity and the development of type 2 diabetes (T2D) (Malik *et al.*, 2010; Basu *et al.*, 2013; Greenwood *et al.*, 2014). Fructose has been suggested as the key component of sucrose and high-fructose corn syrup (HFCS) responsible for the predisposition of the metabolic syndrome (Khitan and Kim, 2013). It has consistently been reported in inducing leptin resistance, fatty liver, insulin resistance and elevated blood pressure (Shapiro *et al.*, 2011; Johnson *et al.*, 2017).

The WHO issued nutritional guidelines to reduce consumption of added sugars to reduce the daily energy intake as well as to reduce the risk of diabetes (IDF, 2014). Accordingly, the use of sweeteners has brought an escalating interest in the management of diabetes, due to their minimal effect in increasing post-prandial blood glucose and caloric value compared to sucrose, glucose and fructose (Mejia and Pearlman, 2019; Antonik *et al*., 2020). Non-nutritive sweeteners (NNS) such as aspartame, saccharin and sucralose are some of the sugar substitutes that are used in commercial products (Chattopadhyay *et al*., 2014). They have low glycemic and insulinemic values compared to sucrose (ADA, 2004). Although NNS are accepted for daily intake, they have life threatening side effects such as cancerous and carcinogenic effects (Shankar *et al*., 2013). Sugar alcohols conversely do not pose any life-threatening adverse effects if taken in acceptable doses. The most frequently used sugar alcohols include xylitol, sorbitol, mannitol, maltitol, lactitol, isomalt and erythritol (Wolever

et al., 2002; Grembecka, 2015b). Studies have suggested that sugar alcohols have the ability to alter the nutritional adequacy of a diet. Since, they have lower energy values compared to easily digestible carbohydrates such as sucrose, glucose and fructose (Wölnerhanssen *et al.*, 2019). Sugar alcohols have a negligible effect on blood glucose and insulin levels compared to sucrose and glucose (Islam, 2011). These sugar alcohols have been reported to have beneficial effects which includes laxative, diuretic, non-cariogenic, antifungal, antibacterial, antioxidant and anti-hyperglycaemic (Awuchi, 2017; Grembecka, 2018; Ibrahim, 2019; Wölnerhanssen *et al.*, 2019). Among these sugar alcohols, the usefulness of xylitol and erythritol as sugar alternatives for diabetes has been extensively studied due to their various health benefits (Wölnerhanssen *et al.*, 2019).

Xylitol has the capacity to lower blood glucose by reducing carbohydrate digestion via inhibiting carbohydrate digesting enzymes activities and intestinal glucose absorption, and increasing muscle glucose uptake (Chukwuma and Islam, 2015). It has also beneficial insulin-sensitizing effects (Salminen *et al.*, 1989; Islam and Indrajit, 2012), improves pancreatic islets morphology (Rahman and Islam, 2014) and the capability to ameliorate diabetes related oxidative stress (Chukwuma and Islam, 2017b). While erythritol has been reported to exhibit postprandial glucose lowering effects, improves glycemic control (Chukwuma, Mopuri, *et al.*, 2018), delays gastric emptying (Wölnerhanssen *et al.*, 2019) and reduces oxidative stress markers (Yokozawa *et al.*, 2002). Although a number of investigations have been conducted on xylitol and erythritol in terms of their antioxidant and antidiabetic properties, comparative research still needs to be conducted to evaluate the comparative and antidiabetic efficacy of these sugar alcohols since these sugar alcohols are not equally sweet and do not have equal calorie content. Therefore, the present study was conducted to investigate comparatively the antioxidant and antidiabetic potential of xylitol and erythritol.

1.2 Literature review

1.2.1 Diabetes mellitus

Diabetes mellitus (DM) is a disorder which was identified by ancient Egyptians, approximately more than 3000 years ago (Lakhtakia and Langerhans, 2013). Araetus of Cappodocia (81-133AD) invented the word “Diabetes” and in 1675, Thomas Willis (Britain) added the word “Mellitus”, as he noted that diabetic urine had a sweet-taste as honey (Ahmed, 2002). The presence of excess sugar in urine and the blood was confirmed in 1776 by Matthew Dobson (Tattersall, 2017). More than 100 years later, Mering and Minkowski (1889) discovered that the pancreas played a role in the pathogenesis of DM and this finding later established the basis of insulin extraction, isolation and purification by Banting and Best (1921) (Ahmed, 2002; Tattersall, 2017).

Insulin is a polypeptide hormone that regulates the stimulation of lipid, carbohydrate and protein metabolism (Wilcox, 2005). It maintains blood glucose levels in the human body by accelerating the uptake of glucose via glucose transporter 4 (GLUT4), into the skeletal muscle and adipocytes (Samuel and Shulman, 2012). Insulin also allows storage of glucose in the form of glycogen in the liver and skeletal muscle and deposition of triglycerides in the adipose tissue (Wilcox, 2005). These actions undertaken by insulin lead to diminished blood glucose levels (Wilcox, 2005; Samuel and Shulman, 2012). However, its insufficiency or action may result in excess glucose production as well as sustained hyperglycemia that defines as DM (Asmat *et al*., 2016).

It has been conceptualized that excess glucose production, also termed as hyperglycemia causes the pathogenesis of DM (Palicka, 2002; Tattersall, 2017). Prolonged hyperglycemia or high blood glucose level affects the polyol pathway, PKC activation, hexosamine pathway, glucose autoxidation, methylglyoxal pathway, methylglyoxal formation and

glycation, and oxidative phosphorylation (Brownlee, 2005; Prabhakar, 2016). These pathways lead to the pathogenesis of related complications such as diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, diabetic cardiomyopathy and diabetic foot diseases (Chawla *et al*., 2016).

1.2.2 Classification of diabetes

Diabetes is a disorder with multifactorial aetiologies, the majority of diabetic patients are classified into one of two categories: type 1 diabetes (T1D), which is also known as Insulin Dependent Diabetes Mellitus (IDDM), and type 2 diabetes (T2D) and also known as Non-Insulin Dependent Diabetes Mellitus (NIDDM) (ADA, 2014; Baynest, 2015). Gestational diabetes is another common type of diabetes that develops in women during their pregnancy (Baynest, 2015). In addition, there are a variety of uncommon types of diabetes, which are caused by drugs, pancreatic destruction, infections, endorcrinopathies and genetic defects, these are included in the 'Other Specific Types' (ADA, 2019; WHO, 2019).

(a) Type 1 diabetes

Type 1 diabetes (T1D), also known as Juvenile type due to its frequent onset in children and young adults (Awuchi *et al*., 2020), results from immune mediated pancreatic destruction of beta cells leading to the loss of insulin production (DiMeglio *et al*. 2018) It is characterized by the presence of islet cell, anti-glutamic acid decarboxylase and insulin antibodies which identify the autoimmune processes that cause beta cell destruction (Baynest, 2015). This condition accounts for approximately 5-10% of diabetic cases (ADA, 2014). There are several pathogenic factors of T1D such as genetic predisposition, autoimmunity, viral infections, environmental and dietary factors (Asmat *et a*l., 2016).

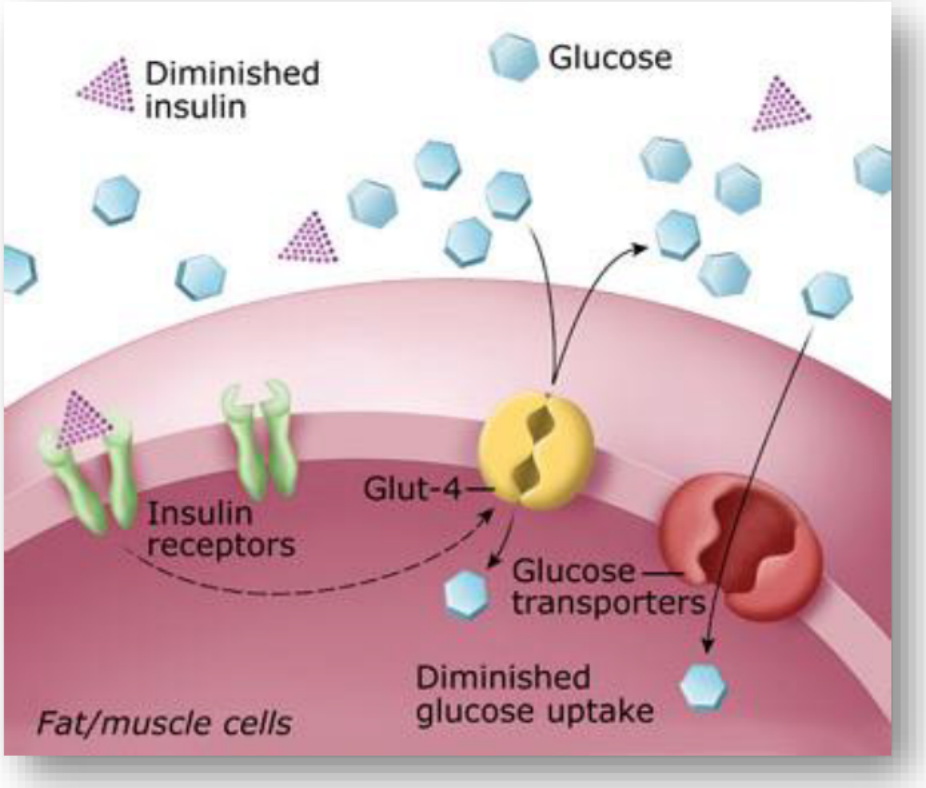

Figure 1.1: Pathophysiology of type 1 diabetes (Adapted without permission from (Kindred Hospitals, 2013))

(b) Type 2 diabetes

Type 2 diabetes (T2D), accounts for 90-95% of total diabetic cases globally (Zheng *et al*., 2017; Pheiffer *et al*., 2018). This disorder occurs among the elderly known as "Adult Type", however over the years it has been exhibited in younger individuals referenced as maturity-onset diabetes of the young (MODY) (IDF, 2019b). This form of diabetes signifies disordered metabolism of proteins, carbohydrates and fat which is triggered by insulin deficiency and reduced sensitivity of insulin to the target tissues (Baynest, 2015). Hyperinsulinism is a condition that prevails due to these defects (Czech, 2018). There are probably many different causes of T2D, however obesity and lifestyle appear to be the triggering factors in its pathogenesis, along with other factors such as genetic predisposition (Asmat, *et al*.,2016; Awuchi *et al*., 2020).

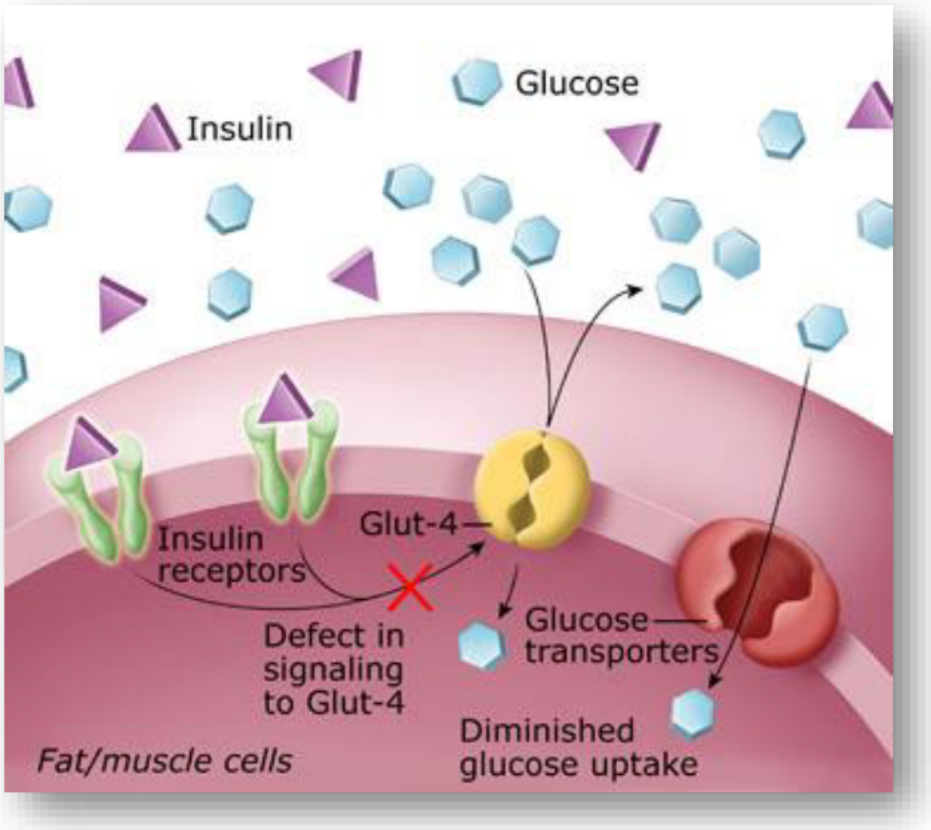

Figure 1.2: Pathophysiology of type 2 diabetes (Adapted from without permission (Kindred Hospitals, 2013))

(c) Gestational diabetes

Gestational diabetes mellitus (GDM) is distinguished as impaired glucose intolerance identified during pregnancy. It develops during the third trimester of pregnancy (McIntyre *et al.*, 2019). This condition affects approximately 2 to 10% of pregnancies which may disappear or advance after delivery (Awuchi *et al.*, 2020). The risk of developing T2DM is more frequent in women with prior history of GDM (Alberti and Zimmet, 1998). The incidence of fasting hyperglycaemia >105 mg/dl in GDM is associated with foetal death. The management of GDM is usually by diet, in some cases insulin therapy is recommended (Awuchi *et al.*, 2020)(ADA, 2003).

1.2.3 Prevalence of diabetes mellitus

(a) Global prevalence of diabetes mellitus

In the year 2000, WHO reported that 151 million people were diagnosed with diabetes globally and it has been estimated to have tripled thus far to 463 million (9.3% of adults aged 20-79 years) (IDF, 2019b). It is projected to increase to 700 million by the year 2045, corresponding to 10.9% of adults aged 20-79 years (Saeedi *et al.*, 2019). Globally, 50.1% of all diabetes cases or 231.9 million people are estimated to be living with undiagnosed diabetes which is a cause for concern (Pheiffer *et al.*, 2018; IDF, 2019b). Over 4 million deaths were reported in 2019 attributed to diabetes alone, imposing a serious threat to global public health (IDF, 2019b). In addition to the global public health burden, diabetes related healthcare expenditure incurs heavy cost on the health systems and individuals (Standl *et al.*, 2019). The diabetes associated healthcare expenditure is anticipated to increase by 8.6% and 11.2% by the year 2030 and 2045, respectively (IDF, 2019b).

(b) Prevalence of diabetes mellitus in Africa

The International Diabetes Federation (IDF) estimated that in 2019 there were approximately 19.4 million adults (20-79 years) who were living with diabetes in Africa. This projection represented a regional prevalence of 3.9% (IDF, 2019b). The proportion of undiagnosed diabetes is the highest in Africa with over 60% of adults currently living with diabetes are unaware of the condition (IDF, 2017; IDF, 2019b). In 2017, diabetes attributed 6% of all mortality in Africa which increased to 6.8% in 2019 (IDF, 2017; IDF, 2019b). Furthermore, 73.1% of deaths attributable to diabetes occurred in individuals under the age of 60 years, thus emphasising the magnitude of the diabetes epidemic (IDF, 2019b; IDF, 2019a). Diabetes is rapidly increasing in this region with projections set to incline by 143% by the year 2045

(IDF, 2019b). However, diabetes still remains one of the largest chronic diseases regardless of the alarming statistics (Asmelash and Asmelash, 2019; Pheiffer *et al.*, 2018).

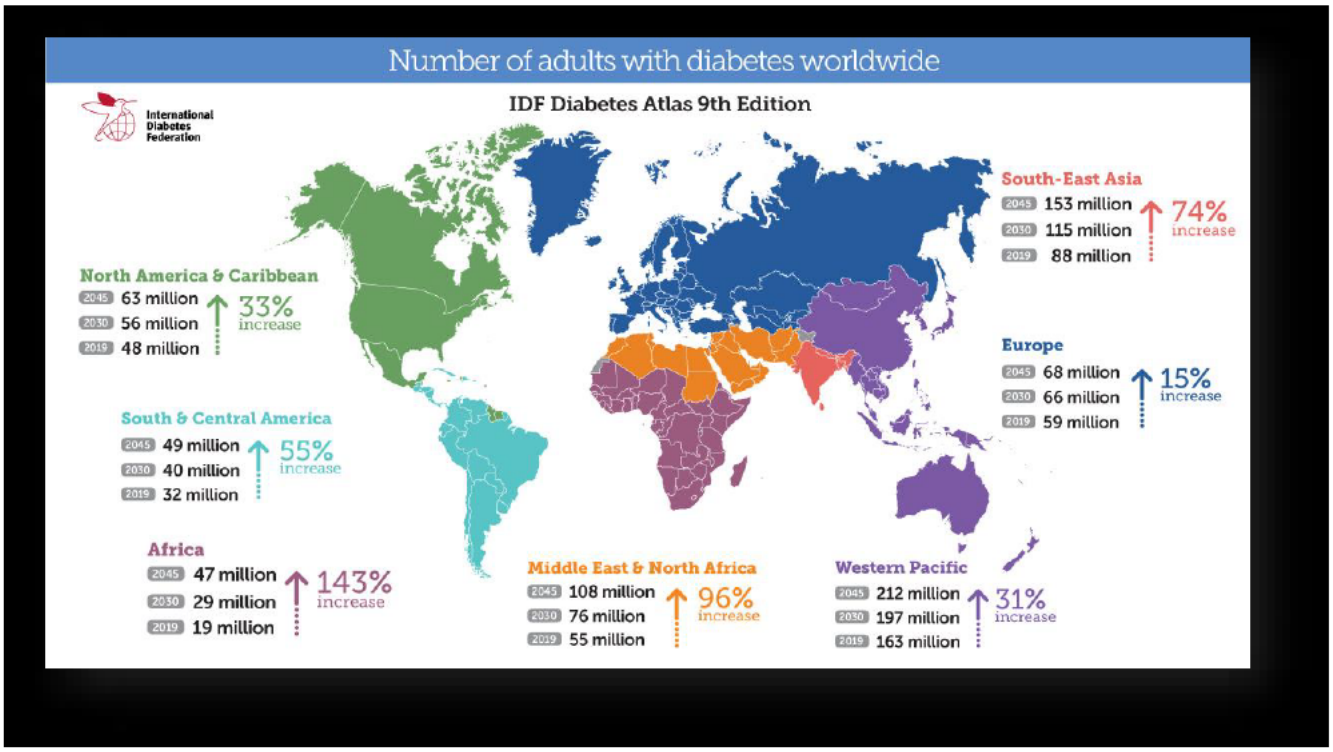

Figure 1.3: Global prevalence of diabetes with 4.7% of the African region diagnosed with diabetes (Adapted without permission from (IDF, 2019b))

(c) Prevalence of diabetes mellitus in South Africa

Historically the burden of diabetes had been significant in developed countries but now a vast increase has been reported in developing countries (Animaw and Seyoum, 2017). These countries often do not have the resources for the prevention, management, diagnosis and treatment of the disease (Kengne *et al.*, 2005). In South Africa, the IDF estimated that 4.6 million people were living with diabetes in 2019, with over 80 000 deaths attributable to diabetes (IDF, 2019b). In 2013, diabetes was the fifth leading cause of death in South Africa which moved up to third and second leading underlying cause of death in 2014 and 2015, respectively (SSS, 2017). In the African region, South Africa has the largest percentage

(23%) of healthcare expenditure due to diabetes (IDF, 2019b), with approximately 2,398.7 people between the ages of 20-79 are living with undiagnosed DM (IDF, 2019c). The magnitude of diabetes burden is further reflected by these cases, causing additional expenditure due to diabetic complications (Dall *et al.*, 2019).

1.3 The complications of diabetes mellitus

The injurious effects of hyperglycemia are separated into microvascular (damage of small blood vessels) and macrovascular complications (damage to large blood vessels) *(Ahmed et al*., 2010; Giacco and Brownlee, 2010). Microvascular complications include diabetic retinopathy, neuropathy and nephropathy while macrovascular complications include diabetic coronary artery disease, cerebrovascular disease or peripheral arterial diseases (Asmat *et al*., 2016; Baynest, 2015). At least 50% people with diabetes suffer from one or more of these complications during the period of their diabetic condition (Deshpande *et al*., 2008).

1.3.1 Microvascular complications

(a) Diabetic neuropathy

Diabetic neuropathy is an impairment of normal activities of the nerves throughout the body which affects sensory, automatic and motor functions (Gérard Said, 2007). The most common form being peripheral neuropathy which causes progressive deterioration of distal nerves of the limbs (Gérard Said, 2007; Ayepola *et al*., 2014). Peripheral neuropathy is thought to develop because of cellular damage to endothelial cells, affecting nerve blood flow and also damage to the neurons affecting conductivity of impulses (Dam, 2002). Signs and symptoms of diabetic neuropathy include numbness, tingling, and some sort of burning or freezing sensation, weakness and loss of reflexes and sensitivities (Soumya and Srilatha,

2011). The prevalence of diabetes related peripheral neuropathy ranges from 16% to 87% (IDF, 2019b) and its responsible for 40% to 60% nontraumatic amputations (Lin *et al*., 2020).

(b) Diabetic retinopathy

Diabetic retinopathy (DR) is a condition that results from the damage of the small vasculature of the retina which leads to visual impairment and blindness (Ayepola *et al*., 2014)(Santos *et al.*, 2011). The vascular lesions that are identified lead to formation of microaneurysms, haemorrhages and increased leakage, which results in retinal edema and lipid exudates (Wang and Lo, 2018). Proliferative retinopathy occurs, causing the development of abnormal blood vessels and fibrous tissue in the retina which results in visual impairment (Santos *et al.*, 2011). Approximately 35.4% of diabetic patients have DR, of which 7.6% have macular edema and a third have vision-threatening DR (Lee *et al*., 2015; IDF, 2017). The global annual incidence of DR ranges from 2.2% to 12.7% and annual progression from 3.4% to 12.3% (Sabanayagam *et al.*, 2019).

(c) Diabetic nephropathy

Diabetic nephropathy is estimated to occur in over half of the people on dialysis or receiving kidney transplants. It is characterized by glomerular basement membrane thickening and arteriosclerosis of small arterioles (Ayepola *et al*., 2014). The alteration in the permeability characteristics of the glomerular capillary wall establishes abnormal albuminuria (Jain, 2012). Microalbuminuria progresses to end-stage renal disease (ESRD) through a number of stages including normoalbuminuria, microalbuminuria and macroalbuminuria (Lewis and Xu, 2008). It has been estimated that more than 80% of ESRD is caused by diabetes or hypertension, or a combination of both (IDF, 2019b).

1.3.2 Macrovascular complications

(a) Diabetic cardiomyopathy

Diabetic cardiomyopathy refers to the existence of abnormal myocardial structure and performance in diabetic individuals in the absence of other cardiovascular disease, such as hypertension, coronary artery disease, valvular, and congenital heart disease (Paolillo *et al.*, 2019)(Jia *et al.*, 2018). A number of mechanisms have been suggested to be involved in the pathophysiology of diabetic cardiomyopathy (Athithan *et al.*, 2019a). Hyperglycemia together with systemic insulin resistance are the major metabolic abnormalities that encourage these mechanisms leading to diabetic cardiomyopathy (Jia *et al*., 2018). Various proposed mechanisms that have been implicated include advanced glycated end-products deposition, a change in substrate metabolism and cardiac lipotoxicity, endothelial and microvascular dysfunction, inappropriate neurohormonal response and oxidative stress (Paolillo *et al.*, 2019; Lee *et al.*, 2019). Clinical trials have shown that the prevalence of diabetic cardiomyopathy ranges from 19% to 26% (Shindler *et al.*, 1996; Rydén *et al.*, 2000; Thrainsdottir *et al.*, 2005), with the mortality rate increasing tenfold over the age of 65 (Athithan *et al.*, 2019b).

(b) Diabetic foot disease

Diabetic foot disease (DFD) is a condition in which foot ulcers form on diabetic individuals (Netten *et al.*, 2020). It is caused by uncontrolled diabetes contributors, mainly diabetic peripheral neuropathy, and peripheral arterial disease (Amin and Doupis, 2016). These contributory factors are present in more than 10% of patients at the time of diagnosis of T2D (Boulton *et al.*, 2005). The risk of amputation in diabetic individuals is 10 to 20 times more common compared to those of non-diabetic individuals (IDF, 2019b). People with DFD are

likely to present with other diabetic complications such as retinopathy, nephropathy, cerebrovascular disease, and ischemic heart disease (Amin and Doupis, 2016).

1.4 Role of oxidative stress in the development of diabetes and its complications

Oxidative stress is the outcome of an imbalance between the production and neutralization of reactive oxygen and nitrogen species (RONS) such that it overwhelms the antioxidant capacity of the cell (Pizzino *et al.*, 2017). These RONS have been implicated in the progression of various disease states, including DM and its associated complications (Alfadda and Sallam, 2012). The overproduction of RONS is triggered by uncontrolled hyperglycemia which may occur via pathways involved in the pathogenesis of diabetic complications: polyol pathway flux, intracellular AGE formation, increased expression of the receptor for AGEs and its activating ligands, protein kinase C activation, and overactivity of the hexosamine pathway (Giacco and Brownlee, 2010; Ayepola *et al*., 2014). The elevation in the production of oxidative products leads to oxidative tissue damage and results in diabetic complications (Chikezie *et al*., 2015).

(a) Increased polyol pathway flux

The polyol pathway is a two-way metabolic pathway, it first reduces glucose to sorbitol which coverts NADPH to $NADP^+$. It than converts sorbitol to fructose which produces NADH from NAD^+ (Giacco and Brownlee, 2010). The co-factor NADPH is utilized to maintain cellular redox cycling which includes the activities of glutathione peroxidase (GPx) and glutathione reductase (GR) which generates glutathione (GSH), a free radical scavenging antioxidant (Chikezie *et al*., 2015). In oxidative stress induced by hyperglycemia, enhanced formation of sorbitol occurs with excessive consumption of NADPH (Yan, 2018). This causes decreased levels of intracellular GSH and GPx, resulting in a suppressed antioxidant

defence system and elevated vulnerability of cells to oxidative damage via oxidative stress (Ayepola *et al*., 2014).

(b) Increased formation of advanced glycation end-products (AGEs) pathway

Advanced glycation end products (AGEs) are formed by the non-enzymatic glycosylation of proteins and/or lipids after exposure to aldose sugar (Giacco and Brownlee, 2010). In diabetic state, the formation of AGEs is elevated in the extracellular matrix and accumulates in sites such as the retina, kidney and atherosclerotic plaques (Brownlee, 1992). AGEs interfere with the normal functions of these sites by fluctuating enzymatic activity, disrupting molecular conformation, and interfering with receptor recognition (Singh *et al.*, 2014). The mechanism by which AGEs alters cell function is via interaction with AGE cell surface receptors of numerous cells including macrophages, neurons, endothelial cells and smooth-muscle cells subsequently activating cell signalling and gene expression that causes oxidative stress and inflammation (Ayepola *et al*., 2014; Ighodaro, 2018).

(c) Increased protein kinase C activation

Protein kinase C (PKC) belongs to a family of protein enzymes that modulate the functions of other proteins by phosphorylation of their serine and threonine amino acid residues (Newton, 2003). There are various isoforms of PKC that function in numerous biological systems, these can be activated by either phosphactidyl serine, calcium or diacylyglycerol (DAG) (Steinberg, 2008; Churchill *et al*., 2009). In a hyperglycemic state, glyceraldehydes-3-phosphate accumulates due to inhibition of glyceraldehyde-3-phosphate dehydrogenase, resulting in enhanced level of dihydroxyacetone-3-phosphate (DHA-3-P) which is a triose isomer of the former (Brownlee, 2001). DHA-3-phosphate is reduced to glycerol-3-phosphate which combines with fatty acids to trigger the de novo synthesis of DAG (Inoguchi *et al.*,

1992). The increased level of DAG in cells up regulates PKC pathway which in turn activates NADPH-oxidases and lipoxygeneses which enhances cellular oxidative damage (Noh and King, 2007).

(d) Increased hexosamine pathway flux

The hexosamine pathway is involved in the metabolism of fructose-6-phosphate (F6P) derived from glycolysis (Giacco and Brownlee, 2010). In this process, the enzyme glucosamine-fructose amidotransferase (GFAT) metabolizes F6P to glucosamine 6-phosphate which is converted to Uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc) (Buse, 2006; Jones *et al.*, 2014). In hyperglycemic condition, excessive amounts of F6P enters into the hexosamine pathway and upregulates the activity of GFAT (Du *et al.*, 2000). This enhances the level of UDP-GlcNAc and in turn increases O-Glucosamine-N-acetyl transferase activity. The increased activity of this enzyme together with hexosamine pathway have been implicated with alterations in gene expression and increased expression of TGF-α and TGF-ß (Lazo-de-la-Vega-Monroy and Fernández-Mejía, 2013; Ighodaro and Akinloye, 2018). These altogether cause the toxic and pro-oxidative role of hexosamine pathway in diabetes and its associated complications (Fantus *et al.*, 2006).

1.5 Management of diabetes mellitus

The management of DM mainly targets glycemic control which aims at relieving symptoms, improving the quality of life of patients, preventing further complications, and reducing mortality (Melmer and Laimer, 2016). Several strategies are available for this purpose: weight loss, physical activity, and dietary modifications, which are the cornerstones of diabetes management (Banerjee *et al*., 2020). Oral therapies and injectable treatments are also required by diabetic individuals due to the progressive nature of the condition (Tahrani *et al*., 2011). Although insulin is the only option of treatment for T1D, the various types of oral

hypoglycemic drugs are still the better choice for the treatment of T2D when changing lifestyle and diet in a positive way makes a big difference in the management of all kinds of diabetes.

1.5.1 Pharmacological treatment

Diabetic pharmacological drugs are biochemical or chemical agents that regulates high blood glucose level in chronic hyperglycemic state (Li *et al.*, 2004; Chaudhury *et al.*, 2017). These drugs are subdivided as follows: drugs that reduce insulin resistance (biguanides and thiazolidinediones), insulin secretagogues and their analogues (sulfonylureas, meglitinides, inhibitors of dipeptidyl peptidase IV (DPP-4) and analogues of glucagon-like peptide-1 (GLP-1)), and drugs that reduce the rate of carbohydrate degradation (alpha-glucosidase inhibitors) (Pontarolo *et al.*, 2020).

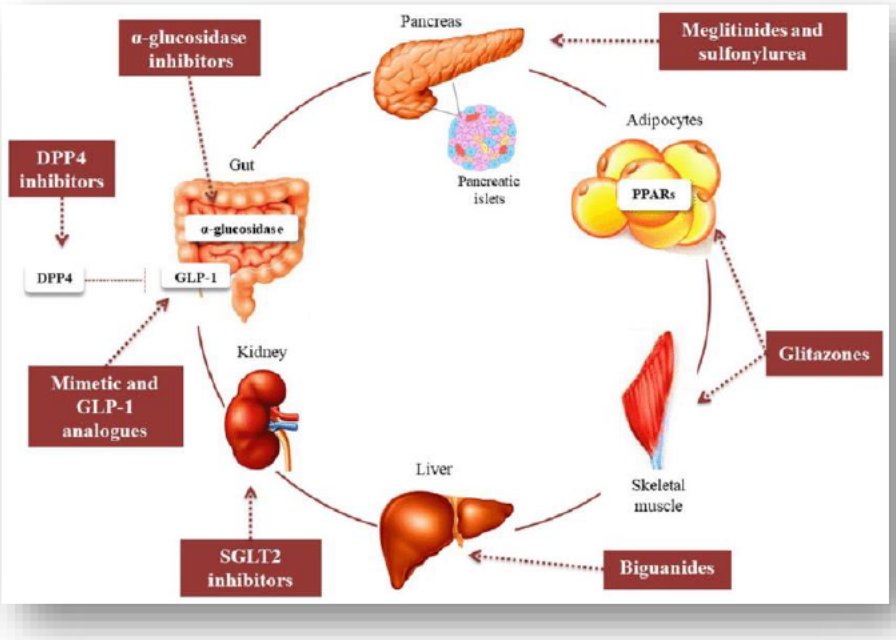

Figure 1.4: Target tissues and mechanism of action of current anti-diabetic drugs

(Adapted without permission from (Pereira *et al.*, 2018))

a. Biguanides

Galega officinalis, is a herbaceous plant that was used in folk medicine to treat diabetes in the middle ages (Tahrani *et al*., 2016). It was found to contain guanidine, galegine, and biguanide which could lower blood glucose levels (Chaudhury, Duvoor, Reddy Dendi, Kraleti, Chada, Ravilla, Marco, Shekhawat, Montales, Kuriakose, Sasapu, Beebe, Patil, Chaitanya K. Musham, *et al.*, 2017). Metformin is a biguanide that has remained as the preferred first-line treatment for T2D (Palmer and Strippoli, 2018). Studies have reported that treatment with metformin in controlling glucose has reduced the risk of diabetes related complications and mortality in diabetic obese patients, and also related to less hypoglycaemic attacks when compared to other antidiabetic drugs (Viollet *et al.*, 2012; Chaudhury *et al.*, 2017). The key function of metformin is to reduce hepatic glucose production by inhibiting the hepatic glucose output. It also improves peripheral insulin sensitivity and increases GLP-1 levels (Tahrani *et al*., 2016; Song, 2016). The mechanism of action of metformin includes AMPK activation in the hepatocytes, stimulated by the inhibitory effect of the drug on the respiratory chain complex I and by blocking adenylyl cyclase via the inhibitory effect on glucagon-induced cAMP production (Viollet *et al.*, 2012). However, metformin has side effects including nausea, weight gain, diarrhoea, abdominal discomfort, and lactic acidosis (Bailey, 2017).

b. Sulfonylureas

Sulfonylureas are a class of antidiabetic agents grouped into three categories: first-generation (chlorpropamide, acetohexamide, tolazamide and tolbutamide), second-generation (glibenclamide, glipizide, gliclazide) and third-generation (glimepiride) (Costello and Shivkumar, 2020). These agents increase the production of insulin by stimulating pancreatic beta cells (Proks *et al.*, 2002). In the ATP-sensitive potassium (K_{ATP}) channel these bind to

SUR subunit therefore closing the channel, resulting in enhanced insulin secretion (Proks *et al.*, 2002). They also limit gluconeogenesis in the liver by decreasing the metabolism of lipids and reducing the clearance of insulin (Harrigan *et al*., 2001). These drugs proliferate the risk of weight gain, cardiovascular disease and hypoglycemia (Costello and Shivkumar, 2020).

c. Thiozolidinediones

Thiazolidinedione's are insulin sensitizers that act on the muscle, adipose and hepatic tissues to increase insulin sensitivity (Yamanouchi, 2010). These agents act on peroxisome proliferator-activated receptor alpha (PPAR-α), causing it to bind to 9-cis retinoic acid receptor (Vielra *et al.*, 2019). The binding regulates genetic transcription and translation of proteins involved in glucose and lipid metabolism. This inevitably causes increased GLUT4 expression which increases glucose uptake in the muscle and adipose tissues (Tyagi *et al.*, 2011). Drugs derived from this class include: troglitozone, pioglitazone and rosiglitazone (Tahrani *et al*., 2016). The drug troglitazone was withdrawn from the market due to its hepatotoxicity (Henney, 2000). The use of rosiglitazone is restricted due to its increased risk of cardiovascular complications. Pioglitazone can be used in renal impairment cases, as it is not associated with hypoglycaemia however it raises concerns regarding fluid retention, peripheral edema and risk of bone fracture in women (Kumar and Murthy, 2016).

d. α- Glucosidase inhibitors

α-Glucosidase inhibitors are a class of drugs that are used in the treatment of T2D, alone or in combination with other antidiabetic drugs. These include acarbose, voglibose and miglitol (Derosa and Maffioli, 2012; Akmal and Wadhwa, 2020). α-Glucosidase inhibitors competitively and reversibly inhibit the intestinal brush border hydrolase enzyme, α-glucosidase (Harrigan *et al*., 2001). This effectively leads to decreased postprandial

carbohydrate absorption, resulting in reduced hyperinsulinism (Akmal and Wadhwa, 2020). Gastrointestinal disturbances are the most commonly reported side effects which include abdominal pain, diarrhoea, and flatulence (Reuser and Wisselaar, 1994).

e. Meglitinides

Meglitinides (nateglinide and repaglinide) are non-sulfonylurea insulin secretagogues which act on the K_{ATP} channel in the pancreas, stimulating the beta cells to release insulin (Pontarolo *et al.*, 2020). These agents share the same mechanism as that of sulfonylureas, they also bind to the sulfonylurea receptor in the β-cells of the pancreas (Chaudhury, Duvoor, Reddy Dendi, Kraleti, Chada, Ravilla, Marco, Shekhawat, Montales, Kuriakose, Sasapu, Beebe, Patil, Chaitanya K. Musham, *et al.*, 2017). However, the action of meglitinide has a short duration and rapid onset hence it lowers the risk of hyperglycemia (Kumar and Murthy, 2016). The major side effect of this class of drug is hypoglycemia (Tahrani *et al*., 2016).

f. Sodium-glucose co-transporters (SGLT2) inhibitors

Sodium-glucose co-transporter (SGLT2) inhibitors are a class of antidiabetics which act by inhibiting renal glucose reabsorption in the kidneys (Abdul-Ghani and DeFronzo, 2014). This results in increased glycosuria and a subsequent reduction in blood glucose levels (Ferrannini and Solini, 2012). Several trials have investigated the safety and efficacy of these inhibitors, displaying body weight reduction, amended glucose control and blood pressure with a low risk of hyperglycemia (Zaccardi *et al.*, 2016). However, there are also potential risks associated with increased glycosuria including genital fungal infections and urinary infections (Komala *et al.*, 2013).

g. Incretin therapies

Incretins are peptide hormones that are secreted from the small intestine endocrine cells in the epithelium, in response to ingestion of glucose (Murohara, 2012). Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) are the main incretins that affect serum glucose levels (Chaudhury, Duvoor, Reddy Dendi, Kraleti, Chada, Ravilla, Marco, Shekhawat, Montales, Kuriakose, Sasapu, Beebe, Patil, Chaitanya K. Musham, *et al.*, 2017). They exert their effects through glucose-dependant stimulation of insulin synthesis and secretion, supressing glucagon release, postponing gastric emptying and increasing satiety (Brunetti and Kalabalik, 2012). Incretins may cause nausea, increased risk of pancreatitis and acute renal failure (Rodbard *et al.*, 2009; Noel *et al.*, 2009; Elashoff *et al.*, 2011)..

h. Dipeptidyl peptidase 4 (DPP-4) inhibitors

Dipeptidyl-peptidase 4 (DPP-4) inhibitors (sitagliptin, saxagliptin and vildagliptin) are a class of hypoglycemic drugs which increase the levels of incretin hormone GLP-1 that assists in maintaining glucose concentrations (Pontarolo *et al.*, 2020). These inhibitors have been reported to have the following adverse effects: upper respiratory tract infection, headache, acute pancreatitis, nasopharyngitis and hypoglycemia (Chaudhury, Duvoor, Reddy Dendi, Kraleti, Chada, Ravilla, Marco, Shekhawat, Montales, Kuriakose, Sasapu, Beebe, Patil, Chaitanya K. Musham, *et al.*, 2017).

1.5.2 Physical exercise

Sedentary lifestyle, or reduced levels of physical activity is often associated with the risk of impaired glucose tolerance, insulin resistance followed by developing T2D (Hu, 2003). The relation between physical inactivity and diabetes is described as the increase in peripheral insulin resistance that occurs as a result of immobility which increases blood glucose levels

(Tomas-Carus *et al*., 2019). Therefore, physical activity is recognized as an effective non-pharmacological therapeutic strategy in diabetic individuals to improve glycemic control, minimize weight gain, reduce blood pressure, cardiovascular risk factors and mortality (Colberg *et al.*, 2016; Hamasaki, 2016). Previous studies have presented compelling evidence on the therapeutic effect of physical activity on diabetic individuals. Aerobic exercise is the generally prescribed type of physical exercise, it has been reported to increase insulin sensitivity (Winnick *et al.*, 2008; Bajpeyi *et al.*, 2009), glycogen synthase activity and GLUT4 protein expression in diabetic patients (Christ-Roberts *et al.*, 2004). Supervised exercise intervention trails found improved glycated hemoglobin (HbA1c), cholesterol and triglycerides, and reduced cardiovascular mortality in diabetic individuals (Sigal *et al.*, 2018).

1.5.3 Dietary management

It is well established that excess energy intake and weight gain in relation to unhealthy diets is associated with developing T2D (Giacco *et al.*, 2013; Ley *et al.*, 2016). As a result, dietary modification forms an essential part for diabetes management (Stephen, 2009). According to the World Health Organization guidelines, adults and children should reduce their daily intake of refined and calorie-containing sugars to less than 10% (IDF, 2014). The high consumption of these sugars is related to health risks which include weight gain, obesity, cardiovascular disease, diabetes and non-alcoholic fatty liver disease (Bray and Popkin, 2014; Rippe and Angelopoulos, 2016). Recently, the use of sugar substitutes such as non-nutritive and nutritive sweeteners has become more prevalent in the management of diabetes (Mejia and Pearlman, 2019; Edwards *et al.*, 2016). These sweeteners have low caloric values compared to conventional sugar, hence are beneficial in the management diabetes (Edwards *et al.*, 2016).

1.5.4 Sugar substitutes

Sweeteners are food additives, which mimic the effect of sugar on taste. Hence, they are termed sugar substitutes (Chattopadhyay *et al*., 2014). These are classified as non-nutritive sweeteners (non-caloric) and nutritive sweeteners (caloric) (Gupta, 2018).

(a) Non-nutritive sweeteners

Non-nutritive sweeteners are sweetening agents which possess lower caloric content and higher sweetening intensity compared to sucrose (table sugar). Obese and diabetic individuals use NNS to reduce carbohydrate and caloric intake for weight management, as well for glycemic control (Lohner *et al.*, 2020). NNS are derived from either natural or chemical sources. For instance, sucralose, aspartame and acesulfame potassium are derived from chemical sources, while stevia and its stevioside extract comes from natural sources (Shwide-Slavin *et al*., 2012; Jarrett and Koh, 2020).

(i) Sucralose

Sucralose was discovered in 1976 by British researchers. It is the only non-nutritive sweetener derived from sugar by a chemical process that replaces three hydroxyl groups with three chlorine atoms (Tandel, 2011; Shwide-Slavin *et al*., 2012). It is 600 times sweeter than conventional sugar (Roberts and Wright, 2002). Sucralose is not recognized by the body as a carbohydrate, it is minimally absorbed and excreted unchanged. As a result, sucralose provides zero calories (Ahmad *et a*l., 2020). The FDA approved the use of sucralose in 15 food and beverage categories in 1998, the broadest initial approval of any food additive (Shwide-Slavin *et al*., 2012).

(ii) Saccharin

Saccharin has the longest history of use compared to other NNS, it was discovered in 1878 by Remson and Fahlberg (Chattopadhyay *et al*., 2014). It is a heterocyclic crystalline compound, which is approximately 300 times sweeter than sucrose (Neltner *et al.,* 2011; Rankin and Poulsen, 2017). Saccharine is considered calorie-free, as it is not metabolized by the body and is excreted unchanged (Ruiz-Ojeda *et al.,* 2019). It is currently approved by the FDA for use in beverages, processed food and as a sugar substitute (Chattopadhyay *et al*., 2014).

(iii) Aspartame

Aspartame was discovered in 1965, it is 200 times sweeter than sucrose (Landrigan and Straif, 2021). Unlike other non-nutritive sweeteners it is completely broken down by the body to its components, which are found in common foods such as milk, vegetables, fruits and meat (Stegink, 1987; Rastogi *et al.,* 2001; Tandel, 2011). In 1981, aspartame was the first sweetener to be approved by the FDA, it is commonly used as a non-cariogenic sweetener (Roberts and Wright, 2002). Aspartame has an ADI of 40 mg/kg body weight (Ruiz-Ojeda *et al.,* 2019).

(v) Acesulfame K

Acesulfame-potassium (Ace-K) was first developed in 1967 by a pharmaceutical company, Hoechst (Shankar *et al*., 2013; Das and Chakraborty, 2016). It is a white crystalline powder which is about 200 times sweeter than sucrose (Shankar *et al*., 2013). Ace-K is used in more than 5000 products in over 100 countries. It was initially permitted in foods including chewing gum, gelatin desserts and sugar free baked goods (Jain *et al*., 2015; Yebra-Biurrum, 2016). In 1988, this high intensity sweetener was approved by the FDA for use in soft drinks

(FDA, 1988). It has an ADI from 0–9 mg/kg to 0–15 mg/kg body weight per day (Das and Chakraborty, 2016).

(b) Nutritive sweeteners

Nutritive sweeteners contain carbohydrates which provide a source of energy (Jain *et al*., 2015). These sweeteners are also referred to as sugars, caloric sweeteners and added sugars (Edwards *et al.*, 2016; Jacob *et al.*, 2016). Sugars are the most common natural products used to produce the desirable sweet taste attribute (Jiao and Wang, 2018) . They are naturally present in the tissues of numerous plants and animal-based products, such as milk (Goldfein and Slavin, 2015). Sugars are comprised of monosaccharides i.e. glucose, fructose and galactose (Jiao and Wang, 2018). These can be chemically combined to produce disaccharides such as lactose, maltose and sucrose (Edwards *et al.*, 2016). Most sugars are considered to provide 4 kcal/g dietary energy (Edwards *et al.*, 2016; Das and Chakraborty, 2016). The main health concern with the intake of sugars is their cariogenic properties (Miano, 2017).

(i) Sucrose

Sucrose is the most commonly used sugar as “table sugar” and reference standard to evaluate the relative sweetness of other sweeteners (Goldfein and Slavin, 2015)(Jiao and Wang, 2018). It is produced commercially by processing sugarcane or sugar beets (Das and Chakraborty, 2016). The white crystal form of sucrose is produced by refinement which removes the brown pigments of unrefined sugar (ADA, 2004). Sucrose is almost completely broken down by digestive enzymes after consumption in the intestinal mucosa to its constituent monosaccharides (glucose & fructose) (Gibson *et al.*, 2013). Glucose causes a glycaemic and insulinaemic response that elicits its uptake into cells, whereas fructose is metabolized in the liver (Gibson *et al.*, 2013)(Laar *et al*., 2020). The excessive consumption of sucrose has been

reported to have a potential influence in the epidemics of the metabolic syndrome, obesity and fatty liver disease (Stanhope, 2016).

(ii) Fructose

Fructose is a simple sugar that is present naturally in fruit and honey, it is a major constituent in two commonly used sweeteners; sucrose and high fructose corn syrup (Jensen *et al.*, 2018). It has a moderately high relative sweetness compared to other nutritive sweeteners (Nabors, 2012). Fructose is widely used for industrial food and pharmaceutical applications (Barclaya *et al.*, 2012). The consumption of fructose is associated with lower increases in plasma glucose and insulin levels compared to other carbohydrate sweeteners (Bantle, 2009). Over the years its use in the diabetic diet has been preferred, however there is concern it may aggravate lipemia, contribute to the prevalence of obesity and a risk of kidney stones (Bantle, 2009; Tappy and Le, 2010). A high flux of fructose to the liver, disturbs glucose metabolism and glucose uptake pathways, and leads to a significantly enhanced rate of de novo lipogenesis and triglyceride synthesis (Jensen *et al.*, 2018; Berná and Romero-Gomez, 2020). These metabolic disturbances appear to underlie the induction of insulin resistance (Balakumar *et al.*, 2016).

(iii) High fructose corn syrup

High-fructose corn syrup (HFCS) is a liquid sweetener composed of fructose and glucose. It was introduced in the 1970s to the food and beverage industry (Buck, 2001). The two most common HFCS commercial products contain 42% fructose and 55% fructose. The balance is primarily glucose and minor amounts of bound glucose (maltose, maltotriose and maltotetraose) (White *et al*., 2015). The use of HFCS has declined over the years, even though it is viewed as safe. This decline may be due to the association of HFCS with obesity and related health risk (Bray *et al*., 2004).

(v) Isomaltulose

Isomaltulose is an isomer of sucrose, its composition of glucose and fructose is linked by α-1,6-glycosidic instead of α-1,2-glycosidic linkage as in sucrose. Due to this digestion process slows down, providing energy in a balanced way and without gastrointestinal distress (Nabors, 2012). Isomaltulose is commercially produced from sucrose by enzymatic rearrangement and it has been used since 1985 in Japan as a sugar replacer (Lina *et a*l., 2002). In 2008, isomaltulose was approved by the FDA as a nutritive sweetener that does not cause tooth decay (Sentko and Ingrid, 2012).

1.5.5 Sugar alcohols

Sugar alcohols (polyols) are chemically defined as saccharide derivatives in which a ketone or aldehyde group is replaced by a hydroxyl group (Moon *et al.*, 2010; Grembecka, 2015b). They are known as caloric/nutritive sweeteners that are white and water-soluble solids which are naturally present in small amounts in some fruits and vegetables and are commercially produced by hydrogenation reaction (Wolever *et al.*, 2002; Awuchi, 2017)(Grembecka, 2015a). Sugar alcohols are extensively used in sugar-free foods and food products such as gum, fruit spread, candy, baked goods and ice cream (Ibrahim, 2019). They are used as sugar substitutes in diabetic food formulations to reduce water activity in 'intermediate moisture foods'; improvement of dehydrated foods; as softeners; and as crystallization inhibitors (Belitz *et al*., 2009). Some sugar alcohols are also widely used in oral and dental care products due to their proven and relevant beneficial effects (Rice *et al.*, 2020; Kõljalg *et al.*, 2020).

Sugar alcohols (**Fig. 1.5**) are classified into three groups according to the number of saccharide units present in the molecule as follows: (i) Monosaccharide-derived sugar alcohols: for example- xylitol, sorbitol and mannitol. These monosaccharides sugar alcohols

are derived from xylose, glucose and mannose respectively; (ii) Disaccharide-derived sugar alcohols: this includes lactitol and maltitol which are derived by the hydrogenation of lactose and maltose, respectively. (iii) Polysaccharide-derived sugar alcohols mixture: this includes Isomalt which is a 1:1 mixture of alpha-D-glucopyranosyl-[1-6]-D-sorbitol (GPS) and alpha-D -glucopyranosyl-[1-6]-D-mannitol (GPM) (Wolever *et al.*, 2002; Grembecka, 2015b; Ibrahim, 2019).

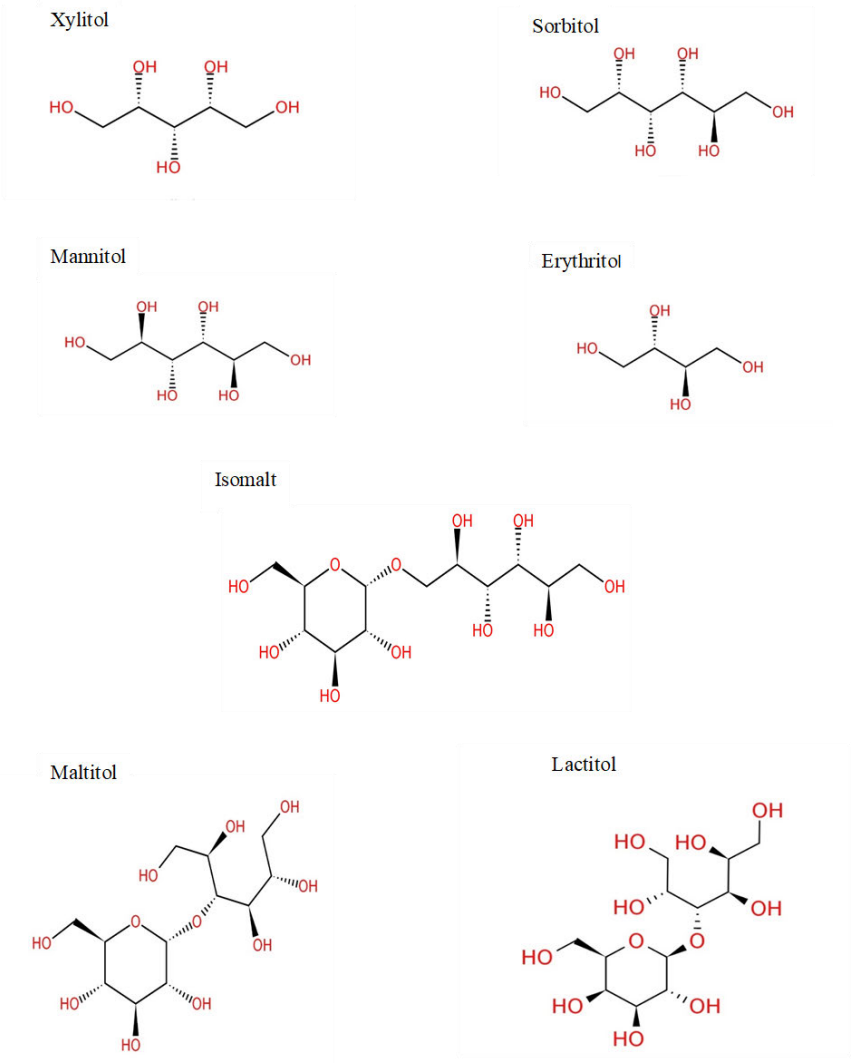

Figure 1.5: Chemical structure of sugar alcohols

(a) Xylitol

Xylitol is a five-carbon crystalline substance occurring naturally in small amounts in many plants, micro-organisms and animal tissues (Mäkinen, 2011; Ur-Rehman *et al.*, 2015). It is produced in humans as part of the hepatic metabolism of carbohydrates at levels of approximately 5-15 g per day (Zacharis, 2012). It is also commercially produced by the hydrogenation of xylose using a nickel-catalyzed reaction process (Mushtaq *et al.*, 2014). The molecule contains a tridentate ligand $(H\text{-}C\text{-}OH)_3$ which reacts with numerous polyvalent cations and oxyacids (Awuchi, 2017). Xylitol is roughly as sweet as sucrose but with moderately lower caloric value of (2.4 kcal/g) compared to sucrose (4 kcal/g), hence it is widely used in foods and pharmaceutical products (Ur-Rehman *et al.*, 2015). Xylitol is partially absorbed in the small intestine by passive diffusion which amounts up to 50% (Grembecka, 2018). About 50-75% of xylitol is passed to the large intestine. Hence, xylitol has lower insulinemic response and glycemic index compared to sucrose (Chukwuma and Islam, 2016). Xylitol has been shown to have beneficial effects including oral health care, lipid metabolism, glycemic control, oxidative stress, weight management and antifungal (Awuchi, 2017; Chukwuma and Islam, 2017b; Grembecka, 2018)

(b) Sorbitol

Sorbitol is a six-carbon alditol also known as D-glucitol. It is naturally present in fruits (peaches, apples, cherries, apricots, nectarines and pears) and some vegetables (Barbieri *et al.*, 2014; Fang *et al*., 2020). It is produced from glucose by catalytic hydrogenation with hydrogen gas and nickel catalyst at high temperatures (Nabors, 2012; Evrendilek, 2012). In alkaline conditions it is produced by electrochemical reduction of dextrose (Barbieri *et al.*, 2014). Sorbitol has sweetness of about 60% of sucrose, with fewer calories (Grembecka, 2015b; Latona and Akinola, 2020). It also characterizes with a 20-fold higher solubility in

water than mannitol (Silveira and Jonas, 2003; Ortiz *et al.*, 2013). Sorbitol has non-cariogenic properties therefore it is used for nutritional purposes in products designated for diabetic people (EFSA, 2011).

(c) Mannitol

Mannitol is six-carbon polyol, an optical isomer of sorbitol which is used as a reserve carbohydrate by some fungi, bacteria and seaweeds (Ruperez and Toledano, 2003; Jacobsen and Frigaard, 2014). It is also naturally found in high amounts in olives, carrots, figs, pineapples, sweet potatoes and larches (Song and Vieille, 2009; Deis and Kearsley, 2012; Ortiz *et al.*, 2013; Chen, Zhang and Wu, 2020). Industrial production of mannitol is based on the catalytic hydrogenation of glucose/fructose derived from invert starch or sugar at high temperatures and pressure (Song and Vieille, 2009; Ghoreishi and Shahrestani, 2009). It has a caloric value of 1.6 kcal/g which is 50% sweet as compared to table sugar, with a desirable cooling effect that is efficient in masking bitter tastes (Grembecka, 2018). This sugar alcohol is non-hygroscopic (Chen *et al*., 2020), which is used as a bulking agent in sugar free coatings and a dusting powder for chewing gum (Grembecka, 2015b). Mannitol is not metabolized by humans therefore it doesn't induce hyeperglycemia so has no insulinemic and glycemic indexes (Livesey, 2012).

(d) Maltitol

Maltitol is a disaccharide polyol (4-O-α-D-glucopyranosyl-D-glucitol), formed from the hydrogenation of maltose to produce an α-1,4 glucose-sorbitol linked polyol (Livesey, 2003; Kearsley and Boghani, 2012; Grembecka, 2015b). It is a crystalline powder that acts as a bulking agent, a stabilizer, an emulsifier, thickener and sweetener (Grembecka, 2015b). Maltitol properties such as sweetness and taste resemble that of sucrose (Deis and Kearsley, 2012). Maltitol is a non-cariogenic agent with applications in many sugar-free foods, as well

as numerous reduced-calorie and reduce-fat foods (Evrendilek, 2012; Kearsley and Boghani, 2012).

(e) Lactitol

Lactitol is disaccharide sugar alcohol obtained from the hydrogenation of lactose using nickel as a catalyst. Its production is composed of galactose and sorbitol (Koivistoinen, 2007;C Zacharis, 2012; Zhang *et al.*, 2020). Lactitol is an odourless white crystalline powder with a sweetness that is 30-40% of sucrose (Koivistoinen, 2007; Ibrahim, 2019). It has an anhydrous form which is applicable in case of moisture-sensitive products. It is used as an emulsifier, thickener and sweetener (Grembecka, 2015b).

(f) Isomalt

Isomalt is a mixture of two isomeric disaccharide alcohols: gluco-sorbitol (α-d-gluco-pyranosyl-1-6-Sorbitol) and gluco-mannitol (α-D-gluco-pyranosyl-1-6mannitol) (Nabors, 2012). It is produced from sucrose in a two-step process, which makes isomalt chemically and enzymatically more stable than sucrose (Gostner *et al.*, 2005; Sentko and Willibald-Ettle, 2012). Isomalt on average has 45–65% sweetness of sucrose. It has synergistic effects when combined with other sugar alcohols or with high-intensity sweeteners, it is also anti-cariogenic and does not increase blood glucose or insulin levels (EFSA, 2011; Grembecka, 2015b). This sugar alcohol is partially digested in the intestines, only supplying half the caloric value of sucrose (Livesey, 2003; Sentko and Willibald-Ettle, 2012).

(g) Erythritol

Erythritol is a four-carbon polyol, that occurs naturally and widely distributed in nature. It occurs as a storage or metabolite compound in fungi and seaweeds, and as a constituent of numerous fruits such as pears, melons and grapes (Moon *et al.*, 2010; Cock *et al.*, 2016). It is

also commercially produced using fermentation in processed vegetables, fermented foods and drinks (Moon *et al.*, 2010). It is a symmetrical molecule, therefore existing in one form, the meso form (Awuchi, 2017). It forms anhydrous crystals with approximately 60-80% sweetness that of sucrose, with no caloric effect and good digestibility without impacting blood glucose and insulin levels (Regnat and Mach, 2018; Rzechonek *et al.*, 2018). Erythritol is rapidly absorbed in the small intestine amounting up to 60-90%, it does not undergo fermentation but it is extracted intact in urine within 24 h (Grembecka, 2018). Its other general features include high stability in acidic and alkaline environments, and high stability against heat (Cock *et al.*, 2016). Erythritol has been found to beneficial effects such as anti-hyperglycemic potential (Chukwuma *et al.*, 2018), oral health care, radical scavenger (Grembecka, 2015b), non-cariogenic and high digestive tolerance (Regnat and Mach, 2018).

1.5.6 Antidiabetic and anti-hyperglycemic properties of sugar alcohols

(a) Xylitol

The consumption of xylitol is accepted for diabetes to help in the management of hyperglycemia as its metabolism is independent of insulin (Grembecka, 2015b; Grembecka, 2018). Numerous studies have been undertaken to examine the effects of xylitol on blood glucose and insulin levels for inclusion in diabetic foods. Mushtaq et al.(2014) reported the ability of xylitol (extracted from mung bean halls) as a supplement for three weeks to reduce serum glucose in normal and diabetic rats with a reduction in food intake and weight gain in a dose dependent manner. Therefore, indicating its glycemic control effect (Mushtaq *et al.*, 2014). The supplementation of 10% xylitol has demonstrated the ability to improve diabetes associated parameters including reduction in blood glucose and serum fructosamine levels, better glucose tolerance in diabetic rats (Islam, 2011; Rahman and Islam, 2014). A study by Chukwuma and Islam (2015) examined the mechanisms behind the anti-diabetic effects of

xylitol using numerous experimental models. It was reported that increasing concentrations of xylitol dose dependently inhibited α-amylase and α-glucosidase enzymes activity *in vitro*. Secondly, in *ex vivo* condition, xylitol dose-dependently decreased intestinal glucose absorption and increased muscle glucose uptake. Furthermore, a bolus dose of xylitol significantly delayed gastric emptying with increased intestinal transit time in non-diabetic and diabetic rats (Chukwuma and Islam, 2015).

The supplementation of xylitol in rats receiving a high-fat diet has been shown to be beneficial in preventing obesity and metabolic abnormalities in a study conducted by Amo et al.(2011). It was observed that xylitol-fed rats compared to high-fat diet-fed rats had significantly lower visceral fat mass and plasma lipid concentrations, with an increase in lipogenic enzymes, fatty acid oxidation and ChREBP (Amo *et al.*, 2011). Xylitol consumption has also been implicated in ameliorating oxidative stress, a factor associated with the exacerbation of diabetes in type 2 diabetic rats (Chukwuma and Islam, 2017b).

In human studies, it has been observed that oral administration of xylitol causes small increase in blood glucose in healthy and diabetic patients (Yamagata *et al.*, 1965; Huttunen, Mäkinen and Scheinin, 1975; Mueller-Hess *et al.*, 1975). Similarly, plasma insulin concentrations do not upsurge (Yamagata *et al.*, 1965) or only moderately increase after the administration of xylitol (Mueller-Hess *et al.*, 1975). Xylitol in comparison to glucose in healthy non-obese men after ingestion had significantly lower increases in plasma glucose and insulin concentrations (Natah *et al.*, 1997). These observations confirm that xylitol does not increase blood glucose to a significant extent. It has also been reported that xylitol has a good tolerance at doses ranging from 20 to 70 g/day (Mäkinen, 2016). A study by Foerster et al. (Foerster *et al*., 1977) reported the good tolerance of xylitol in diabetic children with type 1 diabetes. The study was conducted for four weeks with each child receiving 30 g/day of xylitol (Foerster *et al*., 1977). The tolerance of xylitol was further observed at a higher dose

of 70 g/day in type 1, type 2 and healthy individuals over a period of six weeks (Bonner *et al.*, 1982). The data from these investigations present that xylitol can be safely used as a sugar alternative in diabetic foods.

(b) Sorbitol

The use of sorbitol as a sweetening agent in diabetic foods/diets has received substantial attention since its first recommendation for this purpose in 1929 (Shuman and Kemp, 1956). In 1941, Ellis and Krantz observed that a single dose of 25 or 50 g of sorbitol does not elevate blood glucose levels in normal individuals (Ellis and Krantz, 1941). They further examined the oral administration of 50 g of sorbitol in mild and moderately severe diabetics patients reported that sorbitol did not significantly induce postprandial hyperglycemia (Ellis and Krantz, 1943). A study by Kang et al. (2014) investigated the inhibitory activity of sorbitol against rat intestinal α-glucosidase and porcine pancreatic α-amylase *in vitro*. It was reported that sorbitol possesses an inhibitory effect on these carbohydrate digesting enzymes (Kang *et al.*, 2014). These observations suggest the anti-hyperglycemic activity of sorbitol. Sorbitol has also shown its apparent glycemic control effects *ex vivo* and *in vivo*. It was reported to inhibit glucose absorption in rat jejuna and increased glucose uptake in rat psoas muscle with or without insulin in a concentration dependant manner, *ex vivo*. In normoglycemic and type 2 diabetic rats, sorbitol delayed gastric emptying, enhanced digesta transit, inhibited intestinal glucose absorption and decreased blood glucose levels. Therefore, it was documented that sorbitol has a potential to be used as an anti-hypeglycemic sweetener in diabetic foods and food products (Chukwuma and Islam, 2017a).

(c) Mannitol

Mannitol has glycemic and insulinemic indexes of 0, thus it does not induce hyperglycemia which allows it to be consumed by diabetic individuals (Livesey, 2003; Song and Vieille,

2009). Mäkinen and Hamalainen (1985) studied the effects of the consumption of high amount of mannitol on rat metabolism. The animals were fed a diet that contained 20% mannitol for 8 weeks. The animals presented normal glucose metabolism, associated with lower blood glucose levels and higher liver glycogen levels compared to the control animals. The animals also presented lower insulin secretion and lower serum cholesterol concentrations. Therefore, the metabolism of mannitol can be maintained within normal physiological limits (Mãkinen and Hamalainen, 1985). A study by Dillard et al. (1982) investigated the toxicity of alloxan in rats in relation to pentane and ethane produced during lipid peroxidation induced by i.p. injection of 20 mg of alloxan/100 g b.w. Animals injected with 100 mg of mannitol/100 g b.w (i.p) 30 min prior to alloxan were protected from the peroxidative effects of alloxan which was shown by the reduced production of pentane and ethane. The prevention in the formation of thiobarbituric acid reactive substances in plasma and liver of the animals additionally presented the protective effect of mannitol. Mannitol further reduced the plasma glucose level in the animals even after alloxan injection, presenting its hypoglycemic potential (Dillard *et al*., 1982).

(d) Maltitol

Maltitol is one of the most absorbed and metabolized disaccharide polyols. However, its glucose and insulin response after its consumption is lower as compared to sucrose (Grembecka, 2015b). A study conducted *in vitro* has shown that maltitol is able to exhibit significant inhibition of α-glucosidase and α-amylase activities (Kang *et al.*, 2014). Moreover, maltitol has been reported to inhibit glucose absorption in isolated rat jejunum and increased glucose uptake in isolated rat psoas muscle in the presence of insulin (Chukwuma *et al*., 2017). These observations suggest the use of maltitol for diabetic individuals by

reducing carbohydrate digestion, absorption of glucose and postprandial hyperglycemia (Kang *et al.*, 2014)(Chukwuma *et al*., 2017).

The available data on human studies suggest that maltitol is a good alternative sweetener to sucrose. A single dose of 50 g maltitol led to significantly lower glucose and insulin responses in healthy young subjects compared to sucrose (Secchi *et al.*, 1986). A single oral dose of 30 g or 50 g maltitol in type 2 diabetic subjects also exhibited lower glucose and insulin responses compared to sucrose (Moon *et al.*, 1990; Vessby *et al*., 1990). Additionally, a study by Quilez et al. (2007) examined the consumption of low-calorie muffins containing maltitol compared to conventional plain muffins in non-diabetic healthy subjects. The subjects fed with maltitol showed improved blood glucose, insulin and lipidemic response in comparison to conventionally fed subjects with plain muffins (Quilez *et al*., 2007).

(e) Lactitol

The metabolism of lactitol is unique, once consumed it presents a negligible effect on blood glucose levels. It passes undigested to the colon, without being broken down by any enzymatic activity in the intestine. Henceforth, there is no condition for insulin rendering it appropriate for diabetic individuals (Grembecka, 2015b; Grembecka, 2018). Natah et al. (1997) reported that after the ingestion of 25 g lactitol, the rise in plasma glucose, insulin and C-peptide concentrations were lower compared to ingestion of 25 g glucose in healthy non-obese men. It was suggested that lactitol is a suitable component in the diet for diabetic patients due to its lower glucose and insulin responses (Natah *et al.*, 1997).

A study by Shimomura et al. (2005) examined the effects of consumption of 46 g non-sugar chocolate containing polydextrose and lactitol in place of sucrose and lactose on the concentrations of plasma, insulin and triglycerides in healthy non-diabetic subjects. The non-sugar chocolate had minor effects on the elevation of plasma and insulin concentrations

compared to the control chocolate after ingestion. The serum triglycerides were slightly elevated in non-sugar chocolate subjects; however, this parameter was gradually increased in the control subjects. Additionally, an animal study also presented reduced response of serum triglyceride to the administration of a fat emulsion containing polydextrose and lactitol. These observations suggested that the non-sugar chocolate may have a less effect on body fat deposition (Shimomura *et al.*, 2005).

(f) Isomalt

Over the years the use of isomalt by humans has shown that insulin and blood glucose levels only increase slightly compared to conventional sugar (Thiébaud *et al.*, 1984; Livesey, 2003; Grembecka, 2015b). The impact of isomalt consumption on physiological and metabolic markers with regards to obesity and diabetes was examined in healthy volunteers. It was reported that consumption of milk chocolate containing 70 g isomalt resulted in lower postprandial plasma glucose compared to chocolate containing sucrose. Volunteers that had consumed isomalt presented a reduction in glycation products such as frutosamine and glycated haemoglobin (HbA1c) which are associated in delaying the manifestation of diabetes lesions. Therefore, suggesting its possible benefits for diabetes (Gostner *et al.*, 2005). Holub et al. (Holub *et al.*, 2009) examined the effect of isomalt in patients with T2D for 12 weeks with 30 g of isomalt as an alternative to higher glycemic carbohydrates. The diet was tolerated with significant reductions in fructosamine, glycated haemoglobin, fasting blood glucose, insulin, C-peptide, proinsulin, insulin resistance (HOMA-IR) and oxidized LDL. It was established that 30 g isomalt improved the metabolic control of the diabetic patients significantly (Holub *et al.*, 2009).

(g) Erythritol

Erythritol has been reported to contribute no calories and being well tolerated (good

digestibility) without any impact on blood glucose and insulin levels. Thus making it appropriate for use in diabetic foods and diets (Bornet *et al.*, 1996; Cock *et al.*, 2016). Chukwuma et al. (2018) examined the effect of erythritol on intestinal glucose absorption and muscle glucose uptake in various experimental models. Under *ex vivo* condition, the effect of erythritol was determined by monitoring the glucose concentration change in an incubation medium containing either isolated rat jejunum or psoas muscle at different concentrations (2.5-20%) of erythritol. Erythritol showed an increase in glucose uptake in isolated rat psoas muscle with or without insulin in a dose dependant manner. Insulin significantly improved the effect of erythritol on muscle glucose uptake. Additionally, the effect of an oral dose of erythritol (with phenol red as recovery marker) in normal and T2D rat model was examined on gastric emptying, intestinal glucose absorption, digesta transit and postprandial blood glucose level. Erythritol significantly reduced glucose absorption in the first quarter of the small intestine of normal and diabetic animals. It also slowed down the gastric emptying time in diabetic animals, thus preventing a rise in blood glucose level (Chukwuma *et al.*, 2018).

In a previous study, the supplementation of erythritol in doses of 100, 200, or 400 mg/ kg body weight/ day for ten days in diabetic rats induced with streptozotocin, decreased serum glucose levels significantly with a dose dependant reduction of thiobarbituric acid reactive substances, creatinine and 5-hydroxymethylfurfural in liver, kidney and serum. The study implied that erythritol affects glucose metabolism and reduces lipid peroxidation thus refining oxidative damage involved in the pathogenesis of diabetes (Yokozawa *et al*., 2002).

In a recent human study, Wölnerhanssen et al. (2016) investigated the effect of a single bolus dose of 75 g of erythritol dissolved in 300 mL water given to lean and obese non-diabetic subjects. The acute ingestion of erythritol led to stimulation of gut hormone release (CCK and GLP-1), with significant delay in gastric emptying. Additionally, insulin and plasma glucose concentrations were not affected (Wölnerhanssen *et al.*, 2016). Overduin et al. (2016)

examined the effect of isovolumic meal with partial replacement of sucrose by erythritol in lean and obese subjects. They found lower glucose and insulin levels after erythritol than after sucrose meals. There was no difference in the secretion of GLP-1/PPY levels, subsequent energy intake and sucrose preference between a control meal with sucrose and isovolumic erythritol meals. It was concluded that the satiating effect of the different preloads were comparable (Overduin *et al.*, 2016).

1.6 Study rationale

The emergence of T2D as a global pandemic is one of the major challenges to human health. Dietary modification has been seen to have a huge impact in delaying the progression of the disease. Sugar alcohols namely xylitol and erythritol have shown promising beneficial health effects including the modification of mechanisms involved in the development of T2D. However, the comparative antioxidant and antidiabetic effects of xylitol and erythritol are still not clear. Further investigation is still required to elucidate which sugar alcohol between xylitol and erythritol has the highest potency against T2D.

1.7 Aims and objectives

The aim of this study was to comparatively investigate the antidiabetic and antioxidant potential of xylitol and erythritol using *in vitro*, *ex vivo* and *in vivo* experimental models. The research objectives included the following:

(a) *In vitro* study

- Investigating the antioxidant and inhibitory action of xylitol and erythritol on carbohydrate and lipid digestive enzymes.

(b) *Ex vivo* study

- Investigating the effect of xylitol and erythritol on intestinal glucose absorption in isolated rat jejunum.

- Investigating the effect of xylitol and erythritol on glucose uptake in isolated rat psoas muscle.

(c) *In vivo* study

- Investigating the antidiabetic and antioxidative effects of xylitol and erythritol in a type 2 diabetes model of rats via measuring numerous blood and tissue parameters and histopathological studies.

CHAPTER 2

Materials and methods

2.1 Chemicals and reagents

Food grade Xylitol and Erythritol was purchased from a local supplier (United Scientific Limited, Durban, South Africa). The α-amylase, α-glucosidase, Starch, 3,5-dinitrosalicylic acid (DNSA), Paranitrophenyl α-D-glucopyranoside (PNPG), Mono-basic sodium phosphate, Di-basic sodium phosphate, Potassium ferricyanide, Trichloroacetic acid (TCA), Gallic acid (GA), Sodium nitroprusside, Griess reagent, Triton X-100, 6-hydroxy dopamine (6-HD), Diethylenetriamine-pentaacetic acid (DETAPAC), Glutathione reductase (GR), NADPH, Sodium azide, Ascorbic acid, Sodium hydrogen bicarbonate, Reduced glutathione (GSH), 5,5' – dithiobis – (2-nitrobenzoic acid) (DNTB), Tert-butylhydroxyperoxide (t-BHP), Trizma hydrochloride (Tris-HCl), Oxidized glutathione (GSSG), 1,1'-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich through Merck Chemical Company, Durban, South Africa. Thiobarbituric acid (TBA), Sodium hydroxide (NaOH), Malondialdehyde (MDA), Perchloric acid, Ethylenediaminetetraacetic acid (EDTA), Hydrogen peroxide (H_2O_2), Thiobarbituric acid reactive substance (TBARS), Potassium mono-basic phosphate (KH_2PO_4), Sodium chloride (NaCl), Potassium chloride (KCl), Magnesium sulphate ($MgSO_4$) were purchased from Merck Chemical Company, Durban, South Africa.

2.2 *In vitro* enzyme inhibitory activities

2.2.1 α-Amylase inhibitory activity

The ability of xylitol and erythritol to inhibit α-amylase activity was investigated according to previously described method (Mohamed *et al.*, 2012). Briefly, 1 mL of the different concentrations of xylitol or erythritol (90–720 mM) or standard (positive control) was incubated with equal volume of porcine pancreatic amylase (4 U/mL) in sodium phosphate buffer (20 mM, pH 6.9) for 30 min at 37°C. Then 1 mL of 1% starch solution in sodium phosphate buffer was added to the reaction mixture and incubated for 1 h at 37°C. Finally, 1 mL of 3,5-dinitrosalicylic acid (DNSA) reagent was added into the reaction mixture and boiled for 10 min. Absorbance was measured spectrophotometrically at 540 nm. The reaction without α-amylase was used as a blank, and acarbose (0.037 mM) was used as a positive control. Percentage inhibition was determined according to the following formula:

$$\%\text{Inhibition} = \left[\frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}}\right] \text{x } 100$$

2.2.2 α-Glucosidase inhibitory activity

The ability of xylitol and erythritol to inhibit α-glucosidase activity was measured according to a previously described method (Wu *et al.*, 2012). Briefly, 0.5 mL of the different concentrations of xylitol or erythritol (90–740 mM) or standard (positive conrol) was incubated with equal volume of α-glucosidase (1 U/mL) in an assay buffer (0.1 M sodium phosphate buffer, pH 6.9) for 10 min at 25°C. Then a 0.5 mL of paranitrophenyl-α-D-glucopyranoside was added to the mixture and incubated for 5 min at 25°C. the reaction was stopped with 2 mL of 0.2 M sodium bicarbonate and the absorbance was measured spectrophotometrically at 405 nm. The reaction without α-glucosidase was used as a blank,

and acarbose (0.037 mM) was used as a positive control. Percentage inhibition of α-glucosidase was calculated using the following formula:

$$\%\text{Inhibition} = \left[\frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of Control}}\right] \text{x } 100$$

2.3 *In vitro* antioxidant assays

2.3.1 DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl free radical scavenging effect of xylitol and erythritol was determined according to an established method (Braca *et al.*, 2002). An aliquot of DPPH solution (2 mg in 100 mL MeOH) was prepared, and 2 mL of this solution were added to 2 mL of each sample solution at varying concentrations (90-720 mM). The mixtures were left for 30 min in the dark at room temperature before measuring the absorbance. Ascorbic acid (90-720 mM) was used as a positive control. A spectrophotometer was used to measure the absorbance of each sample or standard at 517 nm. The scavenging activity was calculated using following formula:

$$\text{Scavenging activity } (\%) = 1 - \left[\frac{A_{\text{Sample or standard}}}{A_{\text{Control}}}\right] \text{x } 100$$

2.3.2 Nitric oxide radical scavenging activity

The assay was carried out by a method as described previously (Kurian *et al.*, 2010). Briefly, an aliquot of 50 µL of sodium nitroprusside in phosphate buffer (pH 7.4) was incubated with 100 µL at varying concentrations of xylitol or erythritol or ascorbic acid (90-720 mM) for 3 h at 25˚C. Then 150 µ/L of Griess reagent was added to the reaction mixture and absorbance was read at 540 nm. The scavenging activity was calculated using following formula:

$$\text{Scavenging effect } (\%) = 1 - \left[\frac{A_{\text{Sample or standard}}}{A_{\text{Control}}}\right] \text{x } 100$$

2.3.3 Ferric reducing antioxidant power assay

The reducing power of xylitol and erythritol was determined according to a method as described previously (Benzie, 1996). Briefly, 1 mL of different concentrations (90-720 mM) of xylitol or erythritol was added with 1 mL of 1% potassium ferricyanide and 1 mL of 0.2 M phosphate buffer (pH 6.6). The mixture was incubated at 50˚C for 30 min and thereafter, 1 mL of 10% trichloroacetic acid was added to the mixture. An aliquot of 1 mL of the mixture was mixed with 1 mL distilled water and 200 µL of $FeCl_3$. The absorbance was measured at 700 nm. Ascorbic acid (90-720 mM) was used as a positive control. The ferric reducing power was calculated as follows:

$$\text{Ferric reducing power (\%)} = 1 - \left[\frac{A_{\text{Sample or standard}}}{A_{\text{Control}}}\right] \text{x } 100$$

2.4 *Ex vivo* study

Five adult male Sprague-Dawley rats with mean body weight 180-200 g were procured from the Biomedical Resource Unit located at the Westville Campus of the University of KwaZulu-Natal, Durban, South Africa. The animals were fasted overnight (12 hours) and euthanized using isofor anaesthesia. The abdominal wall was dissected and the whole gastrointestinal tract (GIT) and parts of the psoas muscle were collected and immediately to use for intestinal glucose absorption and muscle glucose uptake study, respectively. All animal procedures were carried out according to the rules and regulations of the Animal Research Ethics Committee of the University of KwaZulu-Natal, Durban, South Africa (Ethical approval number: AREC/037/019D).

2.4.1 Measurement of glucose absorption in isolated rat jejunum

The effect of xylitol or erythritol on intestinal glucose absorption was measured by a method as described previously (Chukwuma and Islam, 2015). Briefly, the jejunal segments of the

collected GIT were flushed with Krebs buffer (118 mM NaCl, 5 mM KCl, 1.328 mM $CaCl_2 \cdot 2H_2O$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$ and 25 mM $NaHCO_3$) and subsequently cut into 5 cm segments. The segments were first inverted to expose the villi and incubated for 2 h with different concentrations (360-1880 mM) of xylitol and erythritol, 0.37 mM acarbose solution (positive control) in 8 mL of Krebs buffer containing 11.1 mM glucose under 5% CO_2, 95% oxygen and 37°C conditions. Glucose with Krebs buffer was used as a control. An aliquot of 1 mL was collected before and after the incubation period. The glucose concentration in the collected samples was measured with an Automated Chemistry Analyzer (Labmax Plenno, Labtest Inc., Lagoa Santa, Brazil). The intestinal glucose absorption was calculated with the following formula:

$$\text{Intestinal glucose absorption} = \frac{GC_1 - GC_2}{\text{Length of jejunum used in cm}}$$

Where GC_1 and GC_2 are glucose concentrations (mg/dL) before and after incubation, respectively.

2.4.2 Determination of glucose uptake in isolated rat psoas muscles

The effect of xylitol or erythritol on glucose uptake in isolated rat psoas muscles was measured according to a method as described previously (Chukwuma and Islam, 2015). Briefly, the collected psoas muscle was immediately rinsed with Kreb's buffer and cut into small pieces of equal weight (0,5 g). Xylitol or Erythritol at varying concentrations (360-1880 mM) were incubated with 0.5 g of the isolated muscle in 8 mL of Krebs buffer containing 11.1 mM glucose under a 5% CO_2, 95% oxygen and 37°C conditions for 2 h in a CO_2 incubator (Lasec, South Africa). Tissues incubated in Krebs buffer with 11.1 mM glucose served as a control. Metformin was used as a positive control. Glucose concentration were measured before and after the incubation period with an Automated Chemistry Analyzer

(Labmax Plenno, Labtest Inc., Lagoa Santa, Brazil). Muscle glucose uptake was calculated with the following formula:

$$\text{Muscle glucose uptake} = \frac{GC_1 - GC_2}{0.5 \text{ g of muscle tissue}}$$

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

2.5 Determination of antioxidative activities in muscle tissue

The remaining muscle tissue after the glucose uptake study was homogenized in 5 mL of ice-cold homogenization buffer (50 mM sodium phosphate buffer with triton X-100, pH 7.5). Homogenates were then centrifuged at 15 000 rpm for 15 min at 40˚C (Eppendorf 5424R, Hamburg, Germany). The supernatants were collected and stored at -20˚C for further analysis.

2.5.1 Induction of oxidative stress in muscle tissue

Equal volumes of each sample tissue homogenate were incubated with 30% volume of pro-oxidant (0.1 mM FeSO4) at 37°C in 5% CO_2 for 30 mins. (O. Erukainure *et al.*, 2017). The incubated samples were analysed for oxidative biomarkers as described below.

2.5.2 Oxidative stress biomarkers

(a) Determination of lipid peroxidation levels

Lipid peroxidation was determined by measuring the concentration of thiobarbituric acid reactive substance (TBARS) as malondialdehyde (MDA) equivalent as described previously (Janero, 1990). Briefly, 50 µL of sample or MDA standard was mixed with an equal volume of 8.1% SDS, 187.5 µL of 20% acetic acid solution, 500 µL of 0.25% thiobarbituric acid (TBA) solution and 212.5 µL of distilled water. The reaction mixture was heated at 95℃ for 1 hour in a water bath. Thereafter, 200 µL of reaction mixture was added into a 96 well plate

and absorbance was read at 532 nm (Synergy HTX Multi-mode reader, BioTek Instruments Inc, Winooski, USA). The level of lipid peroxidation of samples was measured as MDA equivalent by using MDA standard curve.

(b) Determination of reduced glutathione (GSH) level

The level of reduced glutathione(GSH) was estimated by the Ellmen's method (Ellman *et al.*, 1961). Briefly, 300 µL of each sample was precipitated with equal volume of 10% TCA and then centrifuged at 2000 rpm for 10 min at room temperature (25℃) (Hettich Mikro 200 microcentrifuge, Hettich Lab Technology, Tuttlingen, Germany). Thereafter, 80 µL of supernatant, 40 µL of 0.5 mM 5,5' – dithiobis – (2-nitrobenzoic acid) (DTNB) and 200 µL of 0.2 M sodium phosphate buffer (pH 7.8) was added into a 96-well plate. The reaction mixture was incubated for 15 min at 25˚C and the absorbance was read at 415 nm. The level of GSH was calculated from the GSH standard curve.

(c) Determination of superoxide dismutase (SOD) activity

The activity of superoxide dismutase (SOD) was determined by the method as described previously (Gee and Davison, 1989). Into a 96-weel plate, 170 µL of 0.1 mM diethylenetriaminepentaacetic acid (DETAPAC) was added followed by 15 µL of sample or SOD assay buffer (for blank). Thereafter, 15 µL of 1.6 mM 6-HD was added and mixed by gently tapping all four sides of the plate. Absorbance of the reaction mixture was read at 492 nm for 5 mins at 1 min intervals. The enzyme activity was calculated by the formula:

$$\text{Activity} = 1000 \text{ x } \left[\frac{\text{A1} - \text{A2}}{\varepsilon 490}\right] \text{ x } 0.5 \text{ nmol/ min/µg protein}$$

Where ε490 = Molar absorptivity at 490nm = 1.742/mM/cm

A1 and A2 = Reaction rate for sample and blank respectively

(d) Determination of catalase activity

Catalase (CAT) activity was determined by measuring the rate of decomposition of H_2O_2 using a previously described method (Aebi, 1984). Briefly, 340 µL of 50 mM sodium phosphate buffer (pH 7.0) was mixed with 10 µL of the samples, followed by the addition of 150 µL of 2 M H_2O_2. Absorbance was read at 240 nm for 4 min at 1 min interval.

The activity of the catalase enzyme was calculated using the following formula:

$$\text{Activity} = \left(\frac{\text{A}}{\varepsilon 240}\right) \text{x } 0.5 \text{ µmol/min/mg of protein}$$

(e) Determination of glutathione reductase (GR) activity

Glutathione reductase activity was measured by using a method as described previously (Smith, Vierheller and Thorne, 1988). Briefly, 10 µL of sample or glutathione reductase buffer (blank) was added into a 96-well plate, followed by the addition of 221 µL of glutathione reductase assay buffer and 38 µL of 8 mM GSSG solution. Thereafter, 10 µL of NADPH solution was added and the reaction mixture was achieved by gently tapping all four sides of the plate. Absorbance was read at 340 nm for 5 mins in 1-min intervals. Glutathione reductase activity was calculated by the following formula:

$$\text{Activity} = 1000 \text{ x } \left[\frac{\text{A1} - \text{A2}}{\varepsilon 340}\right] \text{ x } 0.5 \text{ µmol/ min/µg ofprotein}$$

Where: ϵ340 = Molar absorptivity at 340 nm = 6.22 mM/cm

A1 and A2 = Reaction rate for sample and blank respectively.

(f) Determination of glutathione peroxidase (GPx) activity

Glutathione peroxidase activity was measured by using a method as described previously with minor changes (Saydama *et al.*, 1997). Briefly, 5 µL of sample or homogenization buffer

(blank) was added into a 96-well plate, followed by the addition of 210 µL of assay buffer, 2.5 µL of 100 mM GSH solution, 2.5 µL of 0.1 U/mL glutathione reductase and 5 µL of distilled water. Thereafter, 2.5 µL of 15 mM NADPH was added and the t-BHP-independent NADPH oxidation at absorbance 340 nm (reaction 1) was read for 8 mins at 2-mins intervals. The reaction was initiated by adding 25 µL of 12 mM t-BHP and absorbance was read at 340 nm (reaction 2) for 6 mins at 2 min intervals. Glutathione peroxidase activity was calculated as follows:

$$\text{Activity} = 1000 \text{ x } \{[\text{A2} - \text{A1}) - (\text{A2b} - \text{A1b})]/\varepsilon 340\} \text{ x } 0.5 \text{ µmol/min/µg of protein}$$

ϵ340 = Molar absorptivity at 340 nm = 6.22/mM/cm

A1 and A2 = Reaction rate for reaction 1 and 2 for sample respectively

A1b and A2b = Reaction rate for reaction 1 and 2 for blank respectively

2.6 *In vivo* glucose uptake study

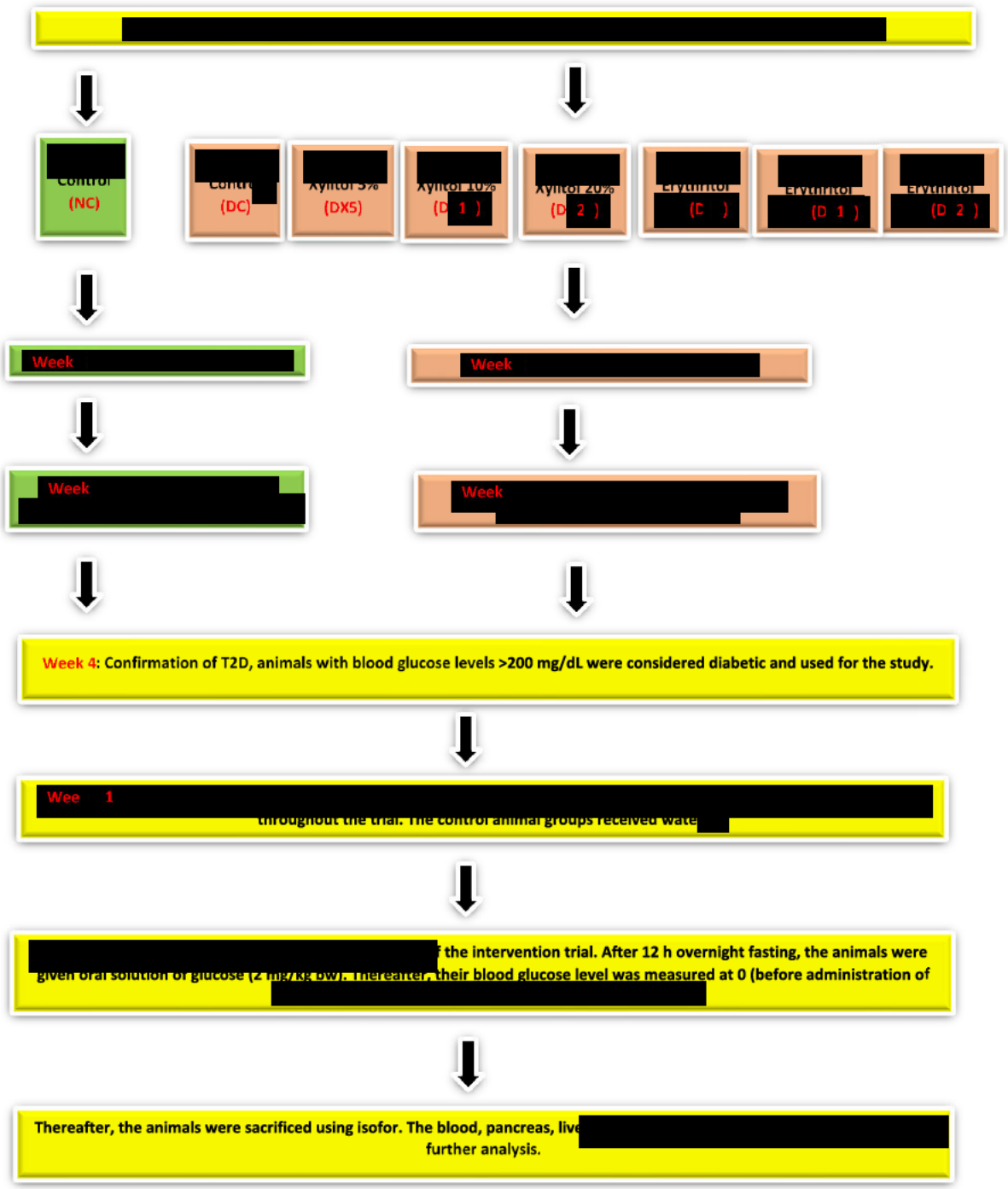

Figure 2.1: Experimental design of the glucose uptake intervention trial on experimental animals.

2.6.1 Animals and grouping

Forty-six seven weeks old male Sprague Dawley rats weighing 180-200 g were procured from the Biomedical Resource Unit (BRU), University of KwaZulu-Natal, Westville campus, Durban, South Africa. Animals were randomly divided into 8 groups as follows: Normal Control (NC) (n=7), Diabetic Control (DC) (n=5), Diabetic Xylitol 5% (DX5) (n=5), Diabetic Xylitol 10% (DX10) (n=6), Diabetic Xylitol 20% (DX20) (n=6), Diabetic Erythritol 5% (DE5) (n=5), Diabetic Erythritol 10% (DE10) (n=6), and Diabetic Erythritol 20% (DE20) (n=6). Animals were maintained according to the rules and regulations of the Experimental Animal Ethics Committee of the University of KwaZulu-Natal, South Africa during the intervention period (Ethical approval number: AREC/037/019D).

2.6.2 Induction of type 2 diabetes

In order to induce type 2 diabetes, the animals in diabetic groups were supplied with a 10% fructose solution in the form of drinking water for the first two weeks to induce insulin resistance, while the animals in the non-diabetic group were supplied with normal drinking water. Thereafter, animals in the diabetic groups were intraperitoneally injected 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, while the animals in non-diabetic groups were injected with a similar volume of citrate buffer only. One week after the streptozotocin injection, the non-fasting blood glucose (NFBG) levels of all animals were measured in the blood collected from the tail vein by using a portable glucometer (Glucoplus, Glucoplus Inc., Quebec, Canada). Animals with NFBG level ≥ 200 mg/dl were considered as diabetic and the animals with NFBG <200 mg/dL or >600 mg/dL were excluded from the study.

2.6.3 Intervention trial

After the confirmation of diabetes, animals in the NC and DBC groups were supplied with normal drinking water and the animals in DX5, DX10 and DX20 groups were supplied with 5%, 10% and 20% xylitol solution respectively, and the animals in the DE10 and DE20 groups were supplied with 5%, 10% and 20% erythritol solution respectively. All animals had free access to rat pellet diet during the entire intervention period unless otherwise indicated. During the 8 weeks intervention period, food and fluid intake were measured daily and the body weight changes and blood glucose levels were measured weekly.

2.6.4 Oral glucose tolerance test (OGTT)

The OGTT was carried out on the last week of the intervention period. In this regard, a single dose of glucose solution (2 g/kg bw) was orally administered to each animal and thereafter blood glucose was measured at 0 (just before glucose ingestion), 30, 60, 90 and 120 min after the glucose ingestion using a portable glucometer (Glucoplus Inc., Quebec, Canada).

2.6.5 Collection of blood and organs

At the end of the intervention period, all animals were humanely euthanized using isofor anaesthesia. Blood was collected via cardiac puncture into sterile plain 15 mL falcon tubes and centrifuged at 3000 rpm for 20 mins to obtain serum, which was stored at -20˚C for further studies. The pancreas, muscle and tissues were collected, washed in 0.9% NaCl, wiped with tissue paper, weighed and preserved at -20°C for further analysis. A small piece of the pancreas of each animal was preserved in a tube containing neutral phosphate buffered formalin at room temperature for histopathological analysis.

2.6.6 Analytical methods

Serum insulin concentration was analysed using a rat insulin ELISA kit (Elabscience, Texas, USA) in multi-plate ELISA reader (Synergy HTX Multi-mode reader, BioTek Instruments Inc, Winooski, USA). Serum lipid profile (total cholesterol, HDL-cholesterol, and triglycerides), aspartate and alanine aminotransferases (AST and ALT), creatine kinase-MB (CK-MB), creatinine, urea and uric acid were measured by an Automated Chemistry Analyzer (Labmax Plenno, Labtest Co. Ltd., Lagoa Santa, Brazil) with commercial assay kits according to manufacturer's manual.

The low-density lipoprotein (LDL) cholesterol level was calculated according to the following formula (Friedewald, Lev and Fredrickson, 1972):

$$\text{LDL} - \text{Cholesterol} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{2.22} + \text{HDL} - \text{Cholesterol}$$

Insulin resistance (HOMA-IR) and β-cell function (HOMA-β) were determined using the following calculations:

$$\text{HOMA} - \text{IR} = \frac{\text{Serum Insulin (UL)x Blood Glucose Level } (\frac{\text{mmol}}{\text{L}})}{22.5}$$

$$\text{HOMA} - \beta \text{ cell function} = \frac{20 \text{ x Serum Insulin (UL)}}{\text{Blood Glucose Level } \left(\frac{\text{mmol}}{\text{L}}\right) - 3.5}$$

2.6.7 Hepatic glycogen content

Hepatic glycogen was measured according to the following method (Lo, Russell and Taylor, 1970) with slight modification. Briefly, 0.5 g of the liver tissue was cut and digested with 0.5 mL of 30% potassium hydroxide saturated with sodium sulphate (Na_2SO_4) and boiled for 30 min. The resulting solution was immediately cooled on ice. Thereafter, 670 µL of 95%

ethanol was added and allowed to stand on ice for 30 min to precipitate the glycogen and centrifuged at 840 g for 30 min. The supernatant was discarded, the glycogen precipitate obtained was dissolved in 1 mL of distilled water and aliquot of 20 µL was taken and made up to 200 µL with distilled water in a test tube. Thereafter, 200 µL of 5% phenol was added to the aliquot and glycogen standards (10, 20, 30, 40, 50, 60, 70, 80, 90 µg/mL) followed by 1 mL of sulphuric acid (96-98%) and subjected to heat in a boiling water bath for 10 min. The tubes were allowed to cool for 10 min and absorbance was read at 490 nm in a multi plate reader (Synergy HTX Multi-mode reader, Bio Tek Instrument Inc. Winooski, USA). Glycogen content was calculated from glycogen standard curve and expressed as µg/mg tissue.

2.6.8 Histopathological analyses

Histological analysis of the pancreatic tissues (preserved in formalin) was carried out according to the standard protocol for paraffin embedding. The tissues were sectioned to 4 µm in slides. Thereafter, p-xylene was used to deparaffinize the slides which were than rehydrated in decreasing ethanol concentration (100%, 80%, 70%, 50%) and rinsed with tap water. Hematoxylin was used to stain the slides for 5 mins, then rinsed with tap water. This was followed by staining with eosin. The slides were mounted in DPX , cover-slipped and viewed with EVOS Floid Cell Imaging Station (Thermo Fisher Scientific – US).

2.6.9 Determination of oxidative stress biomarkers

A 0.5 g from each collected organ tissue was homogenized in 4 mL of homogenizing buffer (50 mM sodium phosphate buffer with triton, pH 7.5). The samples were centrifuged at 15 000 rpm for 10 min at 4˚C. the supernatants were collected and stored at -20˚C for further analysis.

The serum and tissue homogenates were analysed for oxidative biomarkers according to the methods as described in the **section 2.5.2** above.

2.6.10 Determination of purinergic activities

(a) Determination of ATPase activity

This was determined using a previously established method by (Adewoye, Bolarinwa and Olorunsogo, 2000)(O. L. Erukainure *et al.*, 2017) . Briefly, 100 µL of the supernatant was incubated with 100 µL of 5 mM KCl, 650 µL of 0.1 M Tris-HCl buffer, and 20 µL of 50 mM ATP at 37°C in a shaker for 30 mins. 500 µL of distilled water and 1.25% ammonium molybdate were added to the mixture to stop the reaction. 500 µL of freshly prepared 9% ascorbic acid was thereafter added to the reaction mixture and allowed to stand for 30 mins. Absorbance was read at 660 nm.

(b) Determination of ENTDPase

The ENTDPase activity of the hepatic tissues was determined according to the following method (Akomolafe *et al.,* 2017). Briefly, 20 µL of the tissue supernatant was mixed with 200 µL of ENTDPase buffer and incubated for 10 min at 37˚C. 20 µL ATPase was added and incubated for a further 20 min at 37˚C. Thereafter, 200 µL of 10% TCA was added to the mixture and incubated for 10 mins in ice. Absorbance with read at 600 nm.

2.6.11 Determination of acetylcholinesterase activity

The acetylcholinesterase activity of the hepatic tissues was determined by the Ellman's method (Ellman *et al.,* 1961) . Briefly, 20 µL of the tissue supernatant was mixed with 10 µL of 3.3 mM Ellman's reagent (pH 7.0) and incubated with 50 µL of 0.1 M phosphate buffer (pH 8) at 25 °C for 20 mins. 10 µL of 0.05 M acetylcholine iodide was thereafter added to the reaction mixture. Absorbance was read at 412 nm at 3 min intervals.

2.6.12 Determination of lipase activity

Lipase activity of the liver tissues was determined according to the method by (Kim *et al.*, 2010) with slight modifications. Briefly 100 µL of each tissue sample was added to 169 µL of Tris buffer (100 mM Tris–HC1 and 5 mM CaCl2, pH 7.0), 40 µL of porcine pancreatic lipase (2.5 mg/mL in 10 mM MOPS (morpholine propane sulphonic acid) and 1 mM EDTA, pH 6.8) was then added and incubated at 37°C for 15 mins. 5 µL of 10mM p-NPB (p-nitrophenyl butyrate in dimethyl formamide) was added to the reaction mixture and immediately read at 405 nm for 3 mins at 1 min intervals.

2.6.13 Determination of carbohydrate metabolic enzymes activities

(a) Fructose-1,6-bisphosphatase activity

Fructose-1,6-bisphosphatase activity was determined in the muscle homogenate using a previously established protocol (Gancedo and Gancedo, 1971; Balogun and Ashafa, 2017) with slight modification. One hundred microliters of the tissue homogenate were mixed with 1200 µL of Tris–HCl buffer (0.1 M, pH 7.0), 100 µL of fructose (0.05 M), 250 µL 0.1 M MgCl2, 100 µL 0.1 M KCl, and 250 µL 1 mM EDTA. The reaction mixture was incubated at 37°C for 15 mins. The reaction was stopped with 1 mL of 10% TCA. The mixture was then centrifuged for 10 mins at 3000 rpm at 4°C. 100 µL of the supernatant was pipetted into a 96-well plate and incubated with 50 µL of 1.25% ammonium molybdate and freshly prepared 9% ascorbic acid for 20 mins at normal room temperature for colour development. Absorbance was read at 680 nm with a microplate reader and the activity calculated as the amount of inorganic phosphate (Pi) released/min/mg protein.

(b) Glucose- 6 -phosphatase activity

The glucose-6-phosphatase activities of the muscle homogenates were determined according to previously established protocol (Mahato et al., 2011), with slight modification (O. L. Erukainure *et al.,* 2017). Two hundred microliters of tissues were incubated with 100 µL of 0.25 M glucose, 200 µL of 5 mM KCl, 1300 µL of 0.1 M Tris-HCl buffer, and 40 µL of 50 mM ATP at 37°C in a shaker for 30 mins. Thereafter, 1 mL of distilled water and 1.25% ammonium molybdate were added respectively to stop the reaction. 1 mL of freshly prepared 9% ascorbic acid was then added to the reaction mixture and allowed to stand for 30 mins. Absorbance was read at 660 nm and ATPase activity was calculated as the amount of inorganic phosphate (Pi) released/min/mg protein.

(c) Glycogen phosphorylase activity

Glycogen phosphorylase activity was determined in the muscle homogenate as described in section 2.6.13. (b). Glycogen was used as the substrate source (Cornblath *et al.,* 1963; Balogun and Ashafa, 2017).

(d) α- Amylase activity

The muscle homogenate was analyzed for α-amylase activity as describes in **section 2.2.2**. However, the muscle tissue was used as the enzyme source and no standard drug was utilized.

2.7 *In vivo* glucose absorption study

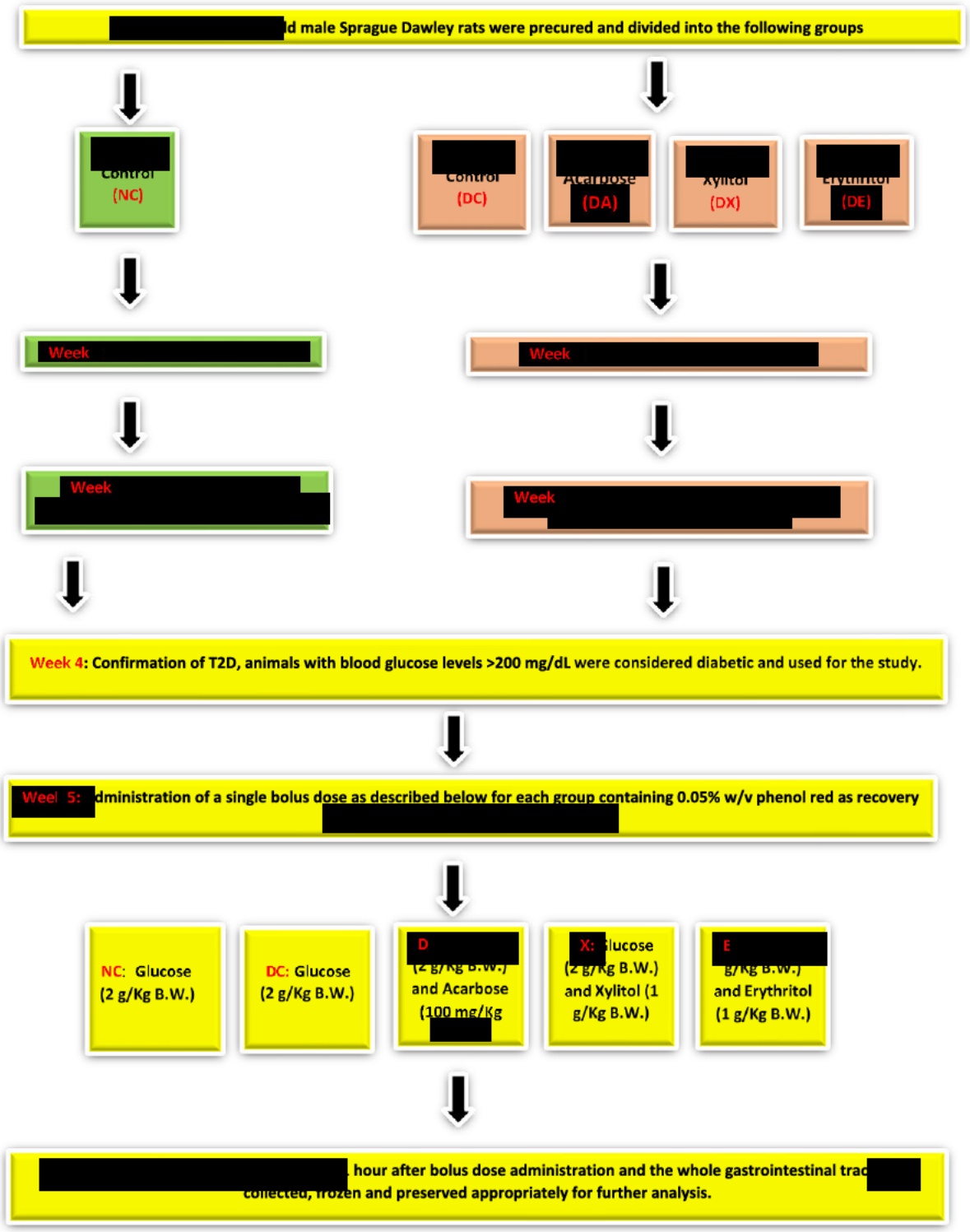

Figure 2.2: Experimental design of the glucose absorption study on experimental animals.

2.7.1 Animals and grouping

Thirty (30) seven weeks old male Sprague Dawley rats weighing 180-200 g were procured from the Biomedical Resource Unit (BRU), University of KwaZulu-Natal, Westville campus, Durban, South Africa. Animals were randomly divided into 5 groups, with six (6) rats in each group as follows: Normal Control (NC), Diabetic Control (DC), Diabetic Acarbose (DA), Diabetic Xylitol (DX), and Diabetic Erythritol (DE). Animals were maintained according to the rules and regulations of the Experimental Animal Ethics Committee of the University of KwaZulu-Natal, South Africa during the intervention period (Ethical approval number: AREC/037/019D).

2.7.2 Induction of type 2 diabetes

Type 2 diabetes was induced and confirmed in the diabetic groups using similar methods described in chapter 2, section 2.6.2.

2.7.3 Feeding and sampling

After the confirmation of diabetes, all animals were fasted overnight (12 h) with free access to drinking water only. Fasting blood glucose (mg/dl) was measured using a portable glucometer (Glucoplus Inc., Quebec, Canada). Thereafter, animals in each group were orally administered the following as a bolus dose containing 0.05% phenol red (PR) as a recovery marker:

- Normal control (NC) group: 2g/kg bw glucose
- Diabetic control (DC) group: 2g/kg bw glucose
- Diabetic acarbose (DA) group: 2g/kg bw glucose + 100 g/kg bw acarbose
- Diabetic xylitol (DX) group: 2g/kg bw glucose + 1 g/kg bw xylitol
- Diabetic erythritol (DE) group: 2g/kg bw glucose + 1 g/kg bw erythritol

The oral bolus dose of each sugar alcohol was chosen considering their gastrointestinal tolerance after ingestion (Livesey, 2003)

The FBG of the animals were measured exactly 1 h after ingestion and without access to food and drinking water. The animals were than sacrificed using isofor, the blood and the entire GIT from each animal was collected. Glucose concentration was measured in the blood samples using an Automated Chemistry Analyzer (LabmaxPlenno, Labtest, Lagoa Santa, Brazil).The GIT from each animal was frozen immediately in liquid nitrogen to prevent the movement of the contents, and then preserved immediately at -80 ˚C for further analysis.

2.7.4 Sample preparation and analysis

The gastrointestinal tract (GIT) from each animal was thawed and divided into eight segments as follows: stomach; 1st, 2nd, 3rd, and 4th quarters of small intestine; cecum; proximal and distal half of the colon. The weight of the segments with the contents and without the contents were weighed to determine the contents weight. The contents and tissues were collected and individually homogenized in ice cold normal saline and centrifuged at 15 000 rpm for 15 min (HettichMikro 200 microcentrifuge, Hettich Lab Technology, Tuttlingen, Germany) (Chukwuma and Islam, 2015).

Phenol red (PR) concentration was determined spectrophotometrically (Synergy HTX Multi-mode reader, BioTek Instruments Inc, Winooski, USA) with bile acid correction in the supernatants of contents and tissue segments according to a previously published method with slight modifications (French *et al.*, 1968). Briefly, a 30 µl of supernatant or phenol red standard (concentrations 0.0038% - 0.00025%) was mixed with 210 µl of 0.1 M dibasic sodium phosphate solution (pH 10.5). The absorbance was read at 560 and 420 nm to correct for bile pigment. The concentration of PR was calculated from the standard curve and

glucose concentration in the intestinal contents was measured using an Automated Chemistry Analyzer (LabmaxPlenno, Labtest Inc., Lagoa Santa, Brazil) using commercial assay kits.

2.7.5 Calculations

The PR (g) and Glucose (g) recovered from each GIT segment was calculated using the following formula:

$$\text{PR recovered (g)} = \text{PR concentration (\%wv)x Homonization volume (mL)}$$

$$\text{Glucose recovered (g)} = \frac{\text{Glucose concentration mg/dl}}{100\ 000}\ \text{x Homegenization volume (mL)}$$

Where, 1 mg/dl ≡ 0.00001 g/ml and 1% w/v ≡ 0.01 g/ml

The calculated PR (g) and glucose recovered (g) were used as indexes to calculate gastric emptying, glucose absorption index (GAI) and digesta transit according to previously described method (Chukwuma & Islam, 2015).

Gastric emptying, denoting the degree of emptying of stomach content was calculated using the following formula:

$$\text{Gastric emptying (\%)} = \frac{\text{A} - \text{B}}{\text{A}}\ \text{x } 100$$

Where, “A” is the total amount of PR (g) recovered from GIT; and “B” is the total amount of PR (g) recovered from the stomach.

Glucose absorption index (GAI) denotes the degree of glucose absorption in each segment of GIT. It is the percentage amount of the glucose absorbed passing through a given segment of GIT, and was calculated using the following formula:

$$\text{Glucose absorption index (\%)in a given segment of GIT} = 1\ \frac{\text{a/b}}{\text{c/d}}\ \text{x } 100$$

Where, “a” is the amount of glucose (g) recovered from that segment; “b” is the amount of phenol red (g) recovered from the same segment; “c” is the amount of glucose (g) given to corresponding animal; and “d” is the amount of phenol red (g) given to the corresponding animal.

Digesta transit in a particular segment of the intestine is the ratio of the amount of content leaving that segment to the amount reaching the same segment. It was calculated in percentage using the following formula:

$$\text{Gigesta transit in a given segment (\%)} = \frac{a}{b} \text{x } 100$$

Where “a” is the amount of phenol red (g) recovered from that particular segment of the GIT to the distal colon excluding the amount of phenol red (g) recovered from that particular segment and “b” is the amount of phenol red (g) recovered from that particular segment of the GIT to the distal colon.

2.8 Statistical analysis

All data are presented as mean ± SD. Data were analysed using a statistical software package (SPSS for Windows, version 25, IBM Corporation, NY, USA), using one-way ANOVA and Tukey’s HSD post hoc test. Values were considered significantly different at $p<0.05$.

CHAPTER 3

Xylitol shows better effects on oxidative stress, intestinal glucose absorption, muscle glucose uptake and carbohydrate digesting enzyme inhibitory activities compared to erythritol: A comparative study

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Preface: This article investigated comparatively the *in vitro* and *ex vivo* antioxidant, as well as the *ex vivo* glucose absorption and uptake effects of xylitol and erythritol. The article is under review in the **Journal of Food and Drug Analysis (D-21-00250)**.

3.1 Abstract

Erythritol and xylitol are two widely used sugar alcohols in various foods and food products due to their numerous health benefits, particularly in terms of diabetes, however no comparative study has been conducted so far in order to understand their comparative efficacy in this regard. The present study was conducted to compare the antioxidant and antidiabetic effects via measuring free radical scavenging activity (DPPH, nitric oxide and ferric reducing power or FRAP) and carbohydrate digesting enzymes inhibitory activity of increasing concentrations of xylitol and erythritol (90-720 mM) *in vitro*. Additionally, the effects of the increasing concentrations of xylitol and erythritol (360-2880 mM) were investigated on intestinal glucose absorption, muscle glucose uptake, lipid peroxidation (LPO) and reduced glutathione (GSH) concentrations, and on the activity of catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR) and glutathione peroxidase (GPx) enzymes in *ex vivo* condition. Xylitol exhibited a greater concentration dependent inhibition of α-amylase and α-glucosidase, with a significantly ($p < 0.05$) better DPPH and NO^- scavenging activity and FRAP compared to erythritol, *in vitro*. In *ex vivo* study, xylitol dose dependently increased muscle glucose uptake, GSH levels and, decreased intestinal glucose absorption and LPO levels compared to erythritol. Xylitol further increased the activity of the antioxidant enzymes more effectively than erythritol. The data of this study suggest that xylitol has better efficacy compared to erythritol in reducing hyperglycaemia and ameliorating diabetes associated oxidative stress compared to erythritol. However, further studies in experimental animals and humans are required to ascertain the findings of this study.

Keywords: Antioxidants, Erythritol, Sugar alcohols, Type 2 diabetes, Xylitol

3.2 Introduction

Diabetes mellitus (DM) has been recognized as a major health issue that has reached alarming levels, with almost half a billion people globally living with diabetes. It is a metabolic disorder defecting carbohydrate, protein and lipid metabolism, resulting in chronic hyperglycaemia (IDF, 2019b). The incapability of pancreatic beta cells to secrete insulin (type 1 diabetes) or the incapability of insulin-sensitive tissues to utilize insulin (type 2 diabetes (T2D)) are the most prominent pathological features of DM. T2D is more predominant than type 1 diabetes and constitutes over 90% of total diabetic cases globally (ADA, 2019). The inability of insulin to exert its role in stimulating glucose uptake into the skeletal and adipose tissue, promotes hyperglycaemia (Zheng *et al.*, 2017). Sidewise to hyperglycaemia, several other factors contribute in the progression of T2D such as oxidative stress leading to a number of diabetes associated complications (Folli *et al.*, 2011). Hence, managing blood glucose level at an optimum level is one of the key factors not only for the better management of diabetes but also to delay or avoid diabetes associated complications (Feinman *et al.*, 2015).

Diabetic patients often try to reduce their calorie intake from refined sugar or sucrose and other natural calorific, low calorific and non-calorific sweetening agents due to their number of short- or long-term side effects on health (Wölnerhanssen and Meyer-Gerspach, 2019). Table sugar or sucrose is still one of the most widely used sweetening agent in the world not only due to its wider availability but also due to lower cost compared to any other available sweetening agents in the market (Goldfein and Slavin, 2015). A recent review reported that the over consumption of sugar or sugar sweetened products is closely linked with the spike of blood glucose, as well as increasing body fats and lipids, body weight and finally developing overweightness, obesity, insulin resistance, impaired glucose tolerance and ultimately T2D (Imamura *et al.*, 2015). Although fructose and high fructose corn syrup are being used as

sugar alternatives, they have also been reported to have similar side effects including obesity and metabolic syndrome (Taskinen *et al*., 2019).

On the other hand, non-nutritive artificial sweeteners are gaining popularity to overweight, obese and diabetic population not only due to their significantly higher sweetness level compared to traditional sweeteners but also due to their low calorific values (Liauchonak *et al.*, 2019). However, concerns have been reported regarding their short- and long-term side effects which are not only limited to the development of obesity but also increasing the risk of diabetes (Daher *et al.*, 2019) There are many other controversial reports available regarding the safety and toxicity of non-nutritive artificial sweeteners (Sharma *et al.*, 2016). Due to above reasons, the use and popularity of less or non-nutritive natural sweetening agents such as sugar alcohols are increasing over above traditional nutritive and non-nutritive artificial sweeteners (Malgorzata Grembecka, 2018).

Sugar alcohols are known for their beneficial roles in modulating insulin release and reducing related factors associated with the metabolic syndrome (Livesey, 2003). Among sugar alcohols, the usefulness of erythritol and xylitol as sugar alternatives has been extensively studied due to a number of their health benefits (Wölnerhanssen *et al.*, 2019). Xylitol has been established to have glucose lowering effects by reducing carbohydrate digestion and intestinal glucose absorption, with an apparent increase in muscle glucose uptake. Animal-based studies indicate that xylitol has beneficial insulin-sensitizing effects, improving glycaemic control and pancreatic islets morphology, and the ability to ameliorate diabetes related oxidative stress (Wölnerhanssen *et al.*, 2019). Erythritol on the other hand has been reported to exhibit an endothelium protective effect, postprandial glucose-lowering effects and improving glycaemic control (Chukwuma, Mopuri, *et al.*, 2018). Moreover, it has been reported to reduce oxidative stress markers such as lipid peroxidation and protein glycosylation in diabetic rats (Yokozawa *et al*., 2002).

Although xylitol and erythritol are widely used as natural sugar substitutes, it is still not clear which one is better between them. Thus, we aimed to investigate comparatively the antioxidant and antidiabetic potential of xylitol and erythritol using some *in vitro* and *ex vivo* experimental models along with some of their underlying mechanism of actions.

3.3 Materials and methods

Kindly refer to Chapter 2; sub sections 2.2-25 and 2.8 for further details.

3.4 Results

3.4.1 Effect of erythritol and xylitol on α-amylase inhibitory activity

The data for alpha amylase inhibitory activity is presented on **Fig. 3.1**. The inhibitory effects of erythritol and xylitol were significantly lower ($p<0.05$) than the standard drug, Acarbose. However, the samples showed concentration dependent inhibitory effect, when xylitol showed higher inhibition compared to erythritol and only significantly ($p<0.05$) so at the highest concentration of 720 mM.

3.4.2 Effect of erythritol and xylitol on α- glucosidase inhibitory activity

The data for alpha glucosidase inhibitory activity is presented on **Fig. 3.2**. A significance difference ($p<0.05$) was observed in the inhibition of α-glucosidase between erythritol and xylitol, with xylitol showing the better activity in a dose dependant manner.

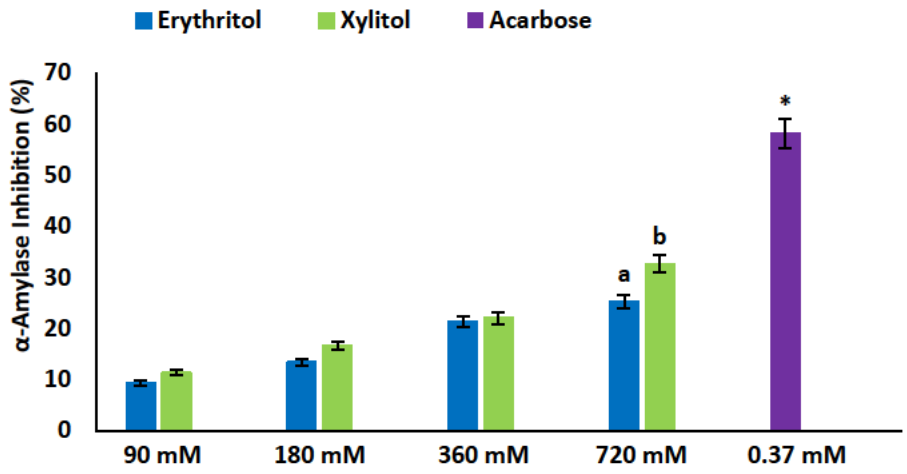

Figure: 3.1. The effect of Erythritol and Xylitol on the activity of alpha amylase *in vitro*. Data are presented as mean ± SD of triplicate analysis. Different letters ("a" - "b") presented above the bars for a given concentration are significantly different from each other; *Significantly different from others ($p < 0.05$. Tukey's HSD post-hoc test, IBM, SPSS, version 25).

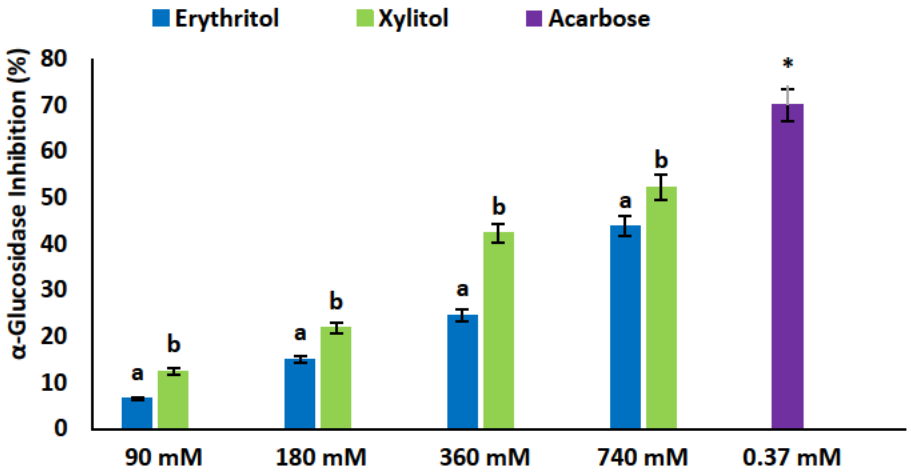

Figure 3.2: The effect of Erythritol and Xylitol on the activity of alpha glucosidase *in vitro*. Data are presented as mean ± SD of triplicate analysis. Different letters ("a" - "b") presented above the bars for a given concentration are significantly different from each other; *Significantly different from others ($p < 0.05$. Tukey's HSD post-hoc test, IBM, SPSS, version 25).

3.4.3 Free radical scavenging activity

The capability of erythritol and xylitol to scavenge DPPH and NO^{-} radicals are presented in **Fig. 3.3a** and **3.3b** respectively. The scavenging activity was concentration dependant for both erythritol and xylitol, it is apparent that the scavenging capability for DPPH radicals was higher than that of the biological radicals NO^{-}. Xylitol showing a significantly higher ($p<0.05$) scavenging effect compared to erythritol.

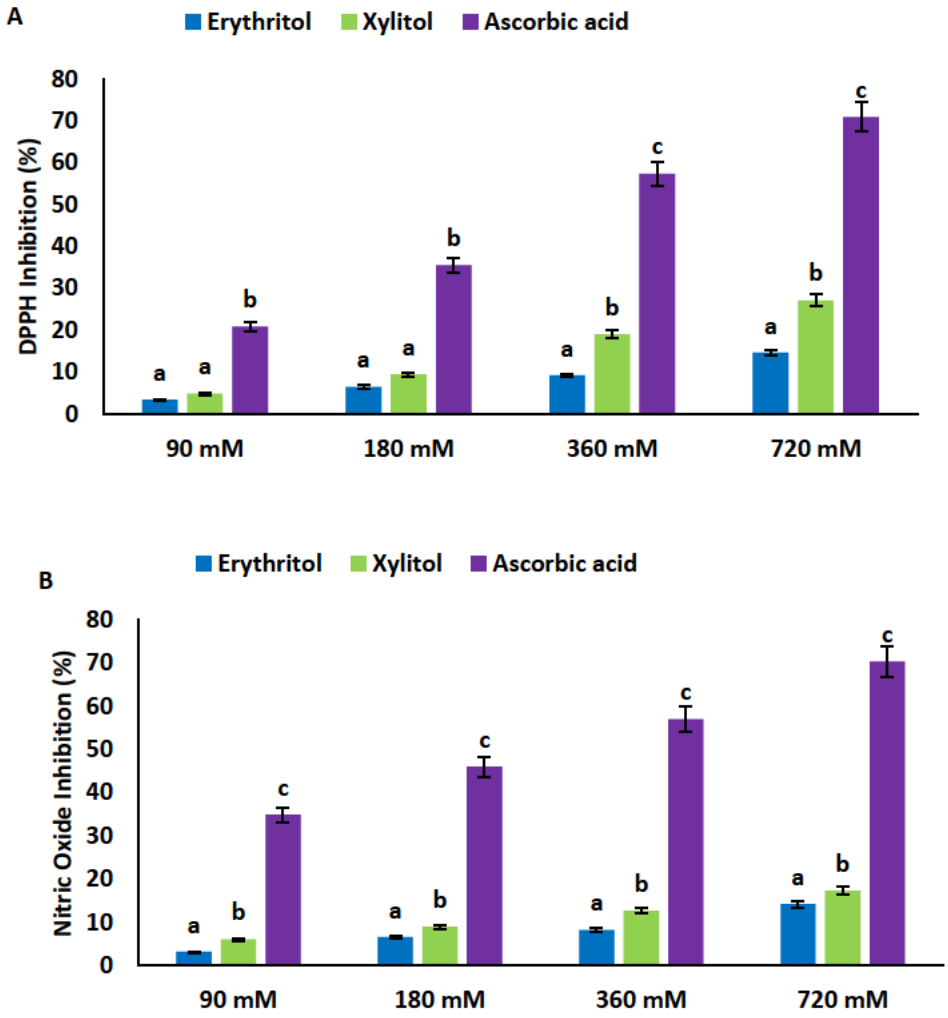

Figure 3.3: The percentage (A) DPPH and (B) Nitric oxide inhibition of Erythritol and Xylitol *in vitro*. Data are presented as mean ± SD of triplicate analysis. Different letters ("a"-"c") presented above the bars for a given concentration are significantly different from each other ($p < 0.05$. Tukey's HSD post-hoc test, IBM, SPSS, version 25).

3.4.4 Ferric reducing antioxidant power (FRAP) of erythritol and xylitol

The data for ferric reducing antioxidant power of erythritol and xylitol are presented in **Fig. 3.4**. There was a moderate increase in FRAP activity by erythritol and xylitol which was significantly ($p<0.05$) lower than the standard drug, Ascorbic acid. However, xylitol showed significantly ($p<0.05$) better activity at 360-720 mM concentrations compared to erythritol.

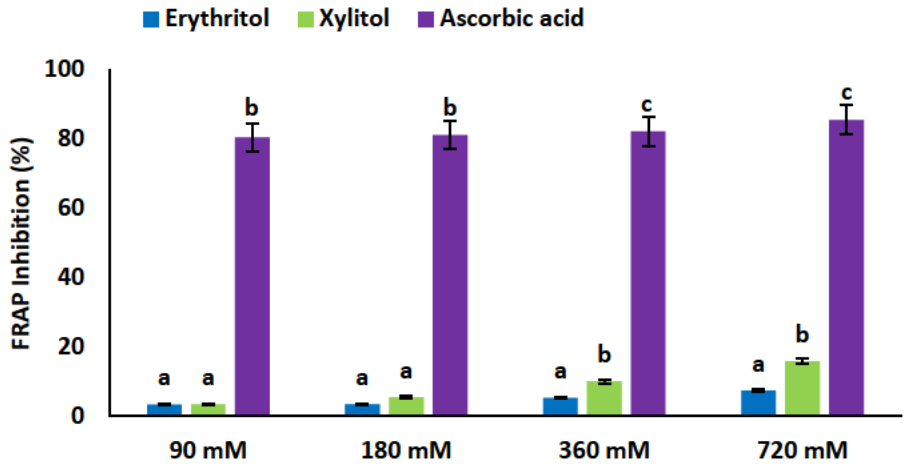

Figure 3.4. The ferric reducing antioxidant power of Erythritol and Xylitol *in vitro*. Data are presented as mean ± SD of triplicate analysis. Different letters ("a"-"c") presented above the bars for a given concentration are significantly different from each other ($p < 0.05$. Tukey's HSD post-hoc test, IBM, SPSS, version 25).

3.4.5 Effects of erythritol and xylitol on glucose absorption in isolated rat jejunum

The data depicting the effects of erythritol and xylitol on glucose absorption in isolated rat jejunum are presented in **Fig. 3.5**. The capacity of glucose absorbed by isolated rat jejunum in the presence of erythritol and xylitol was concentration dependent. Xylitol at 2880 mM

presented the lowest amount of glucose absorbed (1.4 mg/cm jejunum), which was significantly different ($p<0.05$) from the control (5.6 mg/cm jejunum).

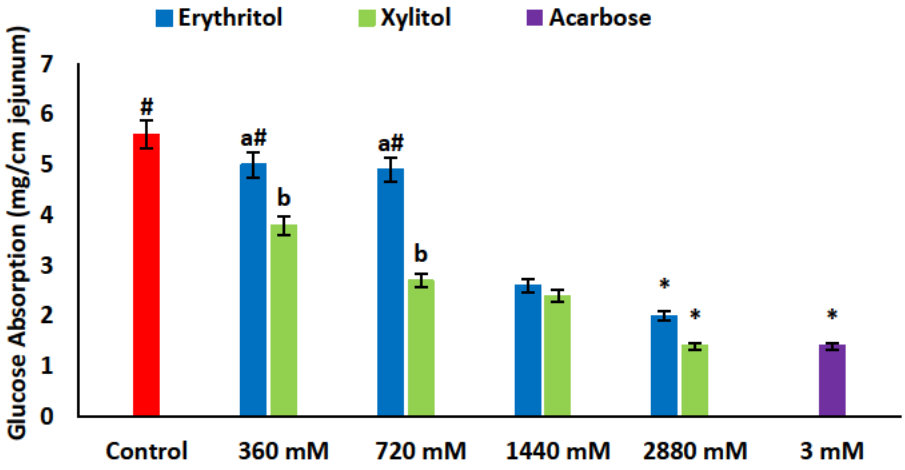

Figure 3.5: The effect of Erythritol and Xylitol on glucose absorption in isolated rat jejunum *ex vivo*. Data are presented as mean ± SD of triplicate analysis. Different letters ("a" and "b") presented above the bars for a given concentration are significantly different from each other. *Significantly different from control. #Significantly different from the standard drug acarbose ($p < 0.05$. Tukey's HSD post-hoc test, IBM, SPSS, version 25).

3.4.6 Effects of erythritol and xylitol on glucose uptake by isolated rat psoas muscle

The data for the effects of erythritol and xylitol on glucose uptake are presented in **Fig. 3.6**. The capacity of glucose uptake by isolated rat psoas muscle in the presence of erythritol and xylitol was concentration dependant. A significant increase ($p<0.05$) compared to the control was observed at 2880 mM for both xylitol and erythritol. Xylitol had a better glucose lowering effect compared to erythritol.

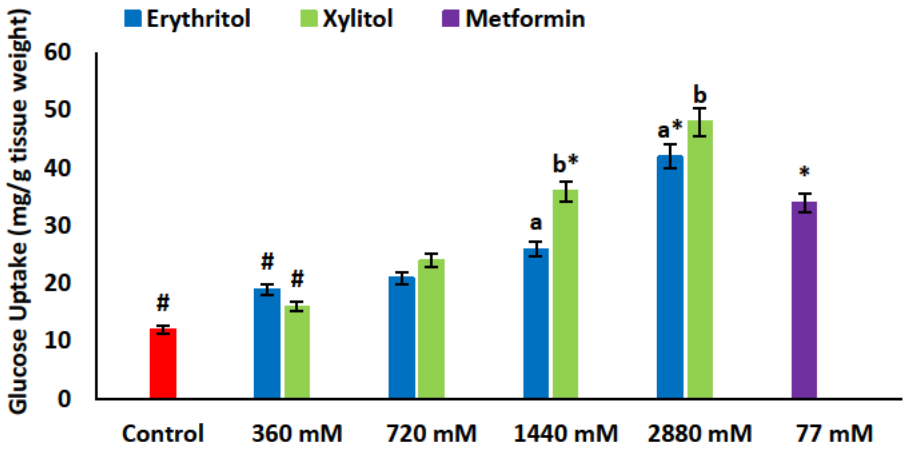

Figure 3.6: The effect of Erythritol and Xylitol on glucose uptake in isolated rat psoas muscle *ex vivo*. Data are presented as mean ± SD of triplicate analysis. Different letters ("a" and "b") presented above the bars for a given concentration are significantly different from each other. *Significantly different from control. #Significantly different from the standard drug acarbose ($p < 0.05$. Tukey's HSD post-hoc test, IBM, SPSS, version 25).

3.4.7 The effect of erythritol and xylitol on Thiobarbituric acid reactive substances (TBARS)

The data for the concentration of TBARS as equivalent of MDA are presented in **Fig. 3.7** . The concentration of TBARS as MDA equivalent was used to determine the level of lipid peroxidation in this experiment. The muscular MDA concentrations after the treatment with xylitol were significantly lower ($p<0.05$) than that of erythritol at 360-2880 mM concentrations.

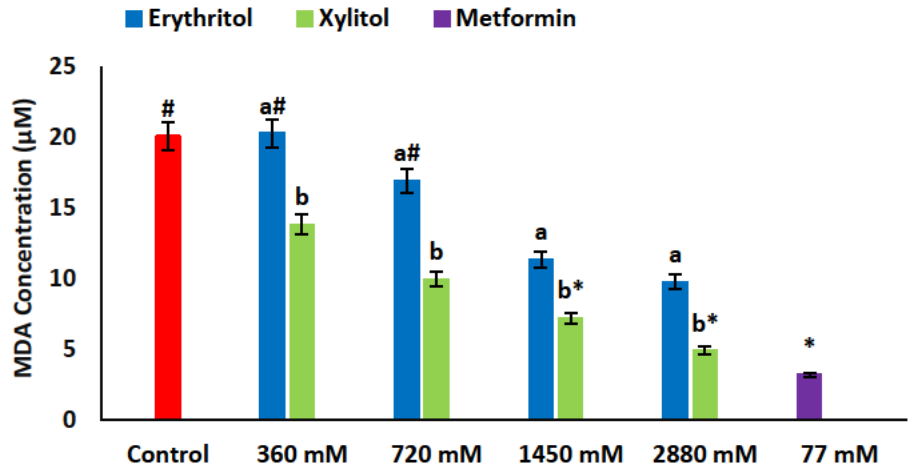

Figure 3.7: The effect of Erythritol and Xylitol on MDA level in tissues with oxidative muscular injury *ex vivo*. Data are presented as mean ± SD of triplicates of analysis. Different letters ("a" and "b") presented above the bars for a given concentration are significantly different from each other. *Significantly different from control. #Significantly different from the standard drug metformin. ($p < 0.05$. Tukey's HSD post-hoc test, IBM, SPSS, version 25).

3.4.8 Effect of erythritol and xylitol on reduced glutathione (GSH) concentration

The data of GSH concentration in the muscular tissue are shown in **Fig. 3.8**. Incubation of muscular tissues with $FeSO_4$ caused a significant ($p<0.05$) reduction in GSH levels denoting oxidative injury. A significance increase ($p<0.05$) was observed for xylitol at 720 to 2880 mM compared to erythritol.

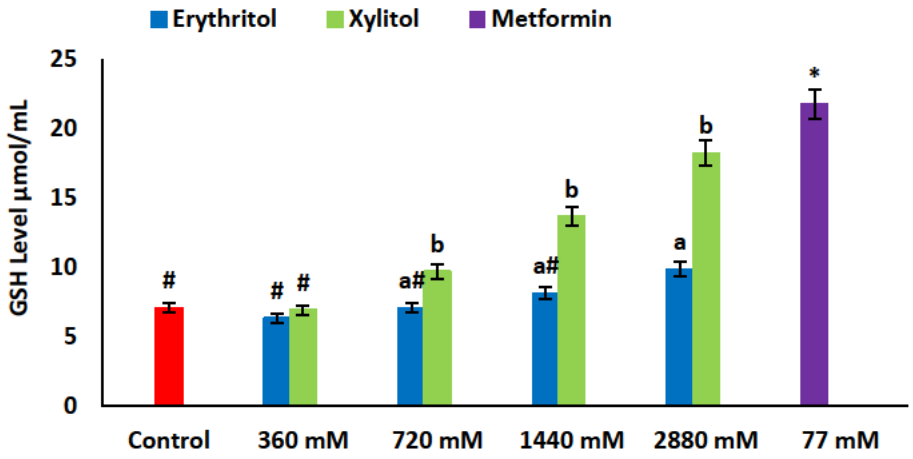

Figure 3.8: The effect of Erythritol and Xylitol on GSH level in tissues with oxidative muscular injury *ex vivo*. Data are presented as mean ± SD of triplicates of analysis. Different letters ("a" and "b") presented above the bars for a given concentration are significantly different from each other. *Significantly different from control. #Significantly different from the standard drug metformin. ($p < 0.05$. Tukey's HSD post-hoc test, IBM, SPSS, version 25).

3.4.9 Effect of erythritol and xylitol on superoxide dismutase (SOD) and catalase (CAT) activities

The data of SOD and CAT activity are presented in **Fig. 3.9a** and **3.9b**, respectively. The SOD activity moderately increased after the treatment with both xylitol and erythritol, with a significantly better increase ($p<0.05$) for xylitol was observed at the highest concentration (2880 mM) compared to erythritol. The catalase activity increased in a dose dependant manner for both xylitol and erythritol, with significantly better increase ($p<0.05$) was observed for xylitol at the highest concentration.

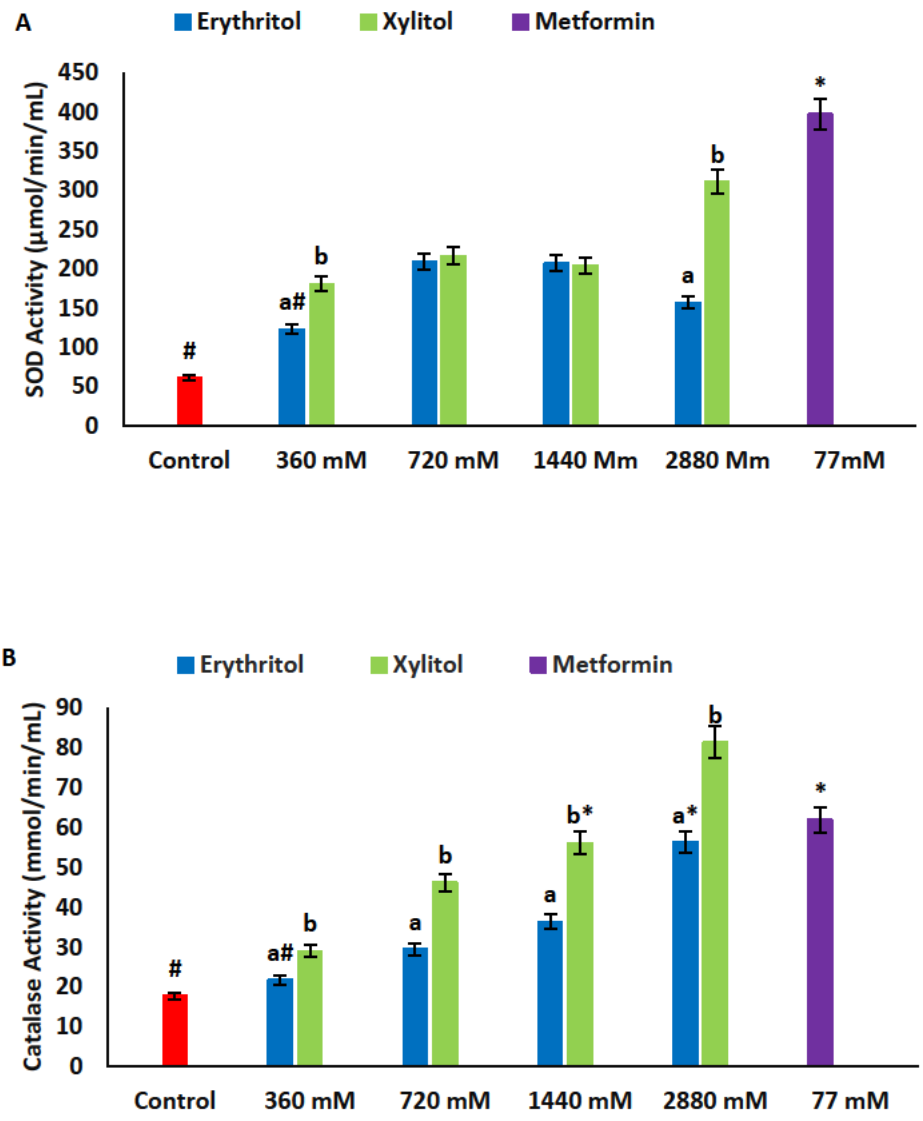

Figure 3.9: The effect of Erythritol and Xylitol on (A) SOD activity and (D) CAT activity in tissues with oxidative muscular injury *ex vivo*. Data are presented as mean ± SD of triplicates of analysis. Different letters ("a" and "b") presented above the bars for a given concentration are significantly different from each other. *Significantly different from control. #Significantly different from the standard drug metformin. ($p < 0.05$. Tukey's HSD post-hoc test, IBM, SPSS, version 25).

3.10 Effect of erythritol and xylitol on glutathione peroxidase and glutathione reductase activity

The data of GPx and GR activity are depicted in **Fig. 3.10** and **3.11** respectively. Treatment with pro-oxidant on muscular tissues reduced GPx and GR activities. A moderate increase in GPx activity was observed upon incubation with erythritol and xylitol, with xylitol presenting a significant ($p>0.05$) increase at the highest concentration 2880 mM. Xylitol also significantly increased ($p<0.05$) GR activity compared to erythritol at higher concentrations, 1440 and 2880 mM (**Fig. 3.11**).

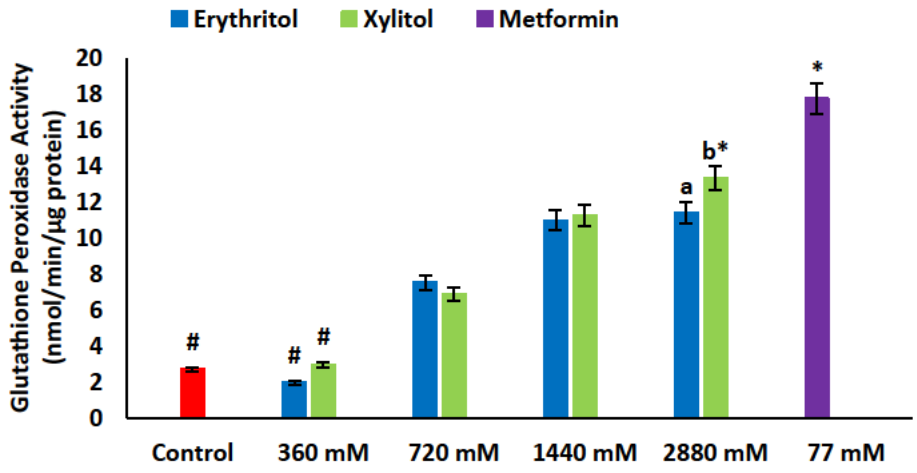

Figure 3.10: The effect of Erythritol and Xylitol on GPx activity activity in tissues with oxidative muscular injury *ex vivo*. Data are presented as mean ± SD of triplicates of analysis. Different letters ("a" and "b") presented above the bars for a given concentration are significantly different from each other. *Significantly different from control. #Significantly different from the standard drug metformin. ($p < 0.05$. Tukey's HSD post-hoc test, IBM, SPSS, version 25).

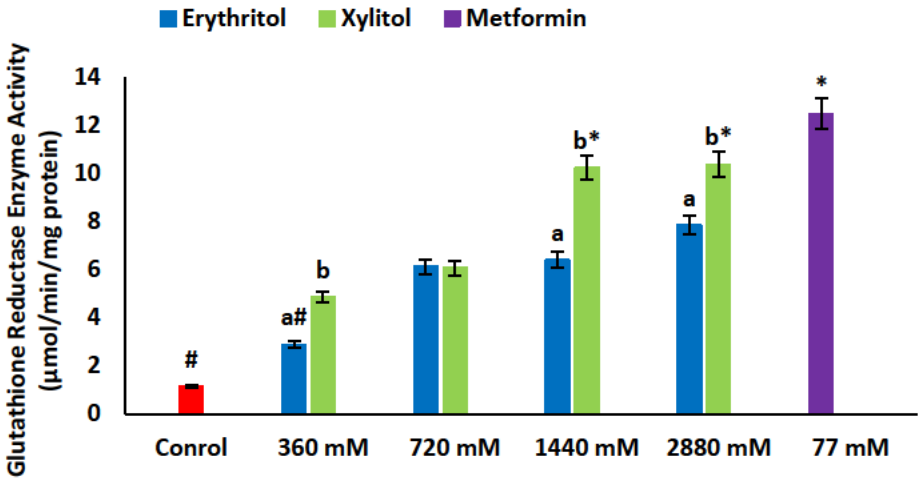

Figure 3.11: The effect of Erythritol and Xylitol on GR activity in tissues with oxidative muscular injury *ex vivo*. Data are presented as mean ± SD of triplicates of analysis. Different letters ("a" and "b") presented above the bars for a given concentration are significantly different from each other. *Significantly different from control. #Significantly different from the standard drug metformin. ($p < 0.05$. Tukey's HSD post-hoc test, IBM, SPSS, version 25).

3.5 Discussion

The use of sugar alcohols as alternative sugar substitutes has increased considerably over the past decade, because of their several health benefits compared to other sweeteners. Erythritol and xylitol are frequently used sugar alcohols in the food industry as they are perceived to have lower caloric values, improved glycemic index and insulinemic response (Grembecka, 2018; Wölnerhanssen *et al.*, 2019). In the present study, erythritol and xylitol were comparatively investigated for their antioxidant and antidiabetic effects *in vitro* and *ex vivo*.

Suppressing the gastrointestinal tract production or absorption of glucose by inhibition of either α-amylase or α-glucosidase enzymes can significantly affect postprandial hyperglycaemia by delaying the breakdown of carbohydrates to glucose. Hence, decreasing the amount of glucose available to the blood stream (Jo *et al.*, 2016). In the present study,

inhibition of α-amylase and α-glucosidase by xylitol was higher than that of erythritol. A previous study has reported the significant inhibitory effect of xylitol on carbohydrate hydrolysing enzymes (Chukwuma and Islam, 2015), thus indicating its antidiabetic potential.

Glucose generated from the breakdown of dietary carbohydrates is predominantly absorbed in the proximal small intestine (jejunal and duodenal regions). Thus, leading to elevated blood glucose levels (Ussar *et al.*, 2017). In the present study, the absorption capacity was seen at its highest in the control sample. However, xylitol presented a significant ($p < 0.05$) reduction in the glucose absorbed from the isolated jejunum compared to erythritol. These results portray the inhibitory potential of xylitol on intestinal glucose absorption.

Disorders of glucose uptake by peripheral tissues are associated to the pathological condition of T2D. This condition is linked to the inability of insulin to exert its role in glucose metabolism, particularly in tissues with high metabolic activity (such as skeletal muscle). Therefore, the skeletal muscle plays a pivotal role in maintaining normal glycaemia in T2D (Abdul-Ghani and Defronzo, 2010). The data from the study reveals that xylitol increased glucose uptake more appreciable than erythritol. This observation further indicates the antidiabetic and hypoglycaemic potential of xylitol.

The acceleration of free radical generation and attenuation of the antioxidant defence system has been implicated in the induction of oxidative stress leading to diabetes (Asmat *et al*., 2016). In this study, the observed dose dependent DPPH and NO^- scavenging capacity of xylitol indicates that its free radical potential is more efficient than that of erythritol. Scavenging capacity of sugar alcohols has been suggested to be dependent on the number of aliphatic hydroxyl group (Kang *et al.*, 2007). Xylitol possesses an additional hydroxyl group compared to erythritol, hence the attributed scavenging capacity. Furthermore, the moderate ferric reducing power of xylitol also portrays its promising antioxidant capability.

Peroxidation of lipids occurs during oxidative stress which generates highly reactive aldehydes such as MDA which upon reaction with hydroperoxides causes damage to cell membranes. MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress (Singh and Kaur, 2014). The observed upsurge of MDA on the incubation with $FeSO_4$ indicates the occurrence of oxidative damage (**Fig. 4a**). Xylitol decreased the levels of MDA at a much higher extent compared to erythritol, signifying its therapeutic activity against oxidative muscular damage.

Glutathione is the most abundant non-protein thiol that serves as a defence mechanism against oxidative stress by detoxifying foreign radicals, participating in amino acid transport and also preventing tissue damage. The reduced levels of GSH have been reported as one of the factors in oxidative tissue damage in T2D (Lutchmansingh *et al.*, 2018). In the present study, reduced GSH levels were observed in the control sample, in the presence of xylitol (720 -1880 mM) the GSH levels were significantly ($p < 0.05$) higher compared to erythritol and control. The results once more portray its beneficial effects in improving antioxidant status.

Superoxide dismutase and catalase are antioxidant enzymes that play an important protective role against cellular and histological damages that are produced by ROS. SOD catalyses dismutation of superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen. Catalase enzymatically processes hydrogen peroxide into oxygen and water, thus neutralizing it. The deficiency of these enzymes has been linked to increased risk of diabetes (Ighodaro and Akinloye, 2018). In the present study, xylitol dose dependently increased SOD enzyme, with a significant increase ($p<0.05$) in CAT enzyme compared to erythritol, thus, suggesting xylitol as a potent antioxidant.

Furthermore, GPx and GR antioxidant enzymes work to maintain normal cellular redox homeostasis. GPx catalyses hydrogen peroxide and organic hydroperoxides reduction using GSH, while GR catalyses the NADPH-dependent reduction of oxidized glutathione resulting from GPx catalysis to form GSH (Yu *et al.*, 2021). In the present study, GPx and GR activities significantly increased ($p<0.05$) for xylitol at its highest concentration compared to erythritol, further denoting its beneficial effects as an ani-oxidant.

3.6 Conclusion

The data from the study suggest that erythritol and xylitol have promising antioxidant and antidiabetic effects *in vitro* and *ex vivo,* with xylitol demonstrating better efficacy compared to erythritol. Hence, xylitol can be used as a preferred dietary supplement compared to erythritol in diabetic foods and food products in order to efficiently reducing hyperglycaemia and ameliorating diabetes associated oxidative stress. Further studies in experimental animals as well as in humans are required to ascertain the findings of this *in vitro* and *ex vivo* studies.

CHAPTER 4

Comparative effects of xylitol and erythritol on modulating blood glucose; inducing insulin secretion; reducing dyslipidemia and redox imbalance in a type 2 diabetes rat model

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Preface: This article investigated comparatively the antioxidative and antidiabetic effects of xylitol and erythritol in type 2 diabetic rats. Will be submitted to an international journal for publication soon.

4.1 Abstract

Xylitol and erythritol have been reported in numerous previous and recent studies as potential antidiabetic sweeteners, however, it is not certain which one is most effective in this regard. In the present study, the effects of xylitol and erythritol were comparatively investigated on blood glucose, insulin level, dyslipidemia, pancreatic islet morphology and β-cell function, and redox imbalance in a type 2 diabetes (T2D) model of rats. Seven-week-old male Sprague-Dawley rats were randomly divided into 8 groups: Normal Control (NC), Diabetic Control (DC), Diabetic Xylitol 5% (DX5), Diabetic Xylitol 10% (DX10), Diabetic Xylitol 20% (DX20), Diabetic Erythritol 5% (DE5), Diabetic Erythritol 10% (DE10), and Diabetic Erythritol 20% (DE20). T2D was induced in the diabetic groups initially by feeding 10% fructose solution to induce insulin resistance followed by an intraperitoneal injection of streptozotocin (40 mg/kg body weight) dissolved in citrate buffer (pH 4.5). The animals in NC group were fed with normal drinking water and injected with citrate buffer only. After the confirmation of diabetes, the xylitol and erythritol with above-mentioned concentrations were supplied to the respective animal groups when the animals in NC and DC groups were supplied with normal drinking water. After 8 weeks intervention period, the body weight, fluid and water intake, blood glucose, serum alanine amino transferase, aspartate aminotransferase, CK-MB and creatinine were significantly decreased, while the serum insulin level, serum lipids, glucose tolerance ability, pancreatic islet morphology and β-cell function, pancreatic and serum redox imbalance were improved the most in the xylitol and erythritol fed groups compared to the DC group, when effects were better for xylitol compared to erythritol. The data of this study suggests that xylitol has better antioxidant and antidiabetic effects compared to erythritol. Therefore, xylitol can be used as a preferrable dietary anti-diabetic sweetener or supplement over erythritol for the management of DM and its associated complications.

Keywords: Xylitol, Erythritol, Sweeteners, Type 2 diabetes, Oxidative stress

4.2 Introduction

Diabetes mellitus (DM) is one of the fastest growing disease in the world, with approximately 463 million people living with diabetes in 2019 (IDF, 2019; Saeedi *et al.*, 2019). This figure has been projected to escalate up to 51% in 2045 (IDF, 2019; Saeedi *et al.*, 2019). DM is a group of metabolic disorders of carbohydrate, protein and lipid metabolism characterized by chronic hyperglycemia (Baynest, 2015). The most common type of DM, type 2 diabetes (T2D) accounts for over 90% of all diabetes cases (ADA, 2019). It results from defective insulin secretion and the inability of the cells to utilize insulin secreted by the pancreatic β-cells, which leads to hyperglycemia (ADA, 2014; Galicia-Garcia *et al.*, 2020). It has been reported that chronic hyperglycemia causes the generation of free radicals which is strongly correlated with the prevalence of T2D (Asmat *et al*., 2016). The increased production of free radicals precedes to redox imbalance leading to oxidative stress (Ayepola *et al.*, 2014). Oxidative stress has been described as the major pathophysiologic factor linking the progression of T2D and its associated complications, both micro- and macrovascular (Giacco and Brownlee, 2010; Ighodaro, 2018). In a number of recent studies, sugar alcohols have been reported to have strong antioxidant effects due to the high number of hydroxyl (-OH) groups in their structures (Kang *et al.*, 2007; Chukwuma and Islam, 2017b; Benedek *et al.*, 2020)

Sugar alcohols are also known for their negligible impact on blood glucose level compared to sucrose, hence, they are used as sugar replacers or sweeteners targeted towards diabetic individuals (Wolever *et al.*, 2002). Among other sugar alcohols, xylitol and erythritol have been extensively used in commercial foods (Wölnerhanssen *et al.*, 2019). They are also found naturally in fruits and vegetables and can be synthesized from their respective

monosaccharides (xylose and erythrose) by catalytic hydrogenation reaction (Grembecka, 2015b). Previous studies have been able to portray beneficial effects demonstrated by both xylitol and erythritol (Salminen *et al.*, 1989; Yokozawa, Kim and Cho, 2002; Chukwuma and Islam, 2015; Chukwuma *et al.*, 2018). In a recent study, low doses (7 to 35 g) of xylitol were able to stimulate the secretion of gut hormones and induce a deceleration in gastric emptying rates (Meyer-Gerspach *et al.*, 2021). Xylitol is effective in reducing the accumulation of visceral fat (Amo *et al.*, 2011), improving diabetic parameters such as blood glucose, serum fructosamine and glucose tolerance (Islam, 2011; Rahman and Islam, 2014). In another study, xylitol indicated to have effective antioxidative potential against T2D-associated oxidative stress (Chukwuma and Islam, 2017b). The replacement of sucrose with erythritol in patients with T2D improved endothelial function and reduced central aortic stiffness (Wölnerhanssen *et al.*, 2021). In a previous study, the supplementation of erythritol in doses of 100, 200, or 400 mg/kg bw/day in diabetic rats, reduced serum glucose levels significantly with a decrease in thio-barbituric acid reactive substances, creatinine and 5-hydroxymethylfurfural in liver, kidney and serum (Yokozawa *et al.*, 2002). Erythritol has been reported to exert anti-hyperglycemic effects by reducing intestinal glucose absorption, increasing muscle glucose uptake, improving glucose metabolic enzymes activity and enhancing Glut-4 and IRS-1 expression in diabetic animals (Chukwuma, Mopuri, *et al.*, 2018).

These studies indicate the emerging evidence of the beneficial effects of xylitol and erythritol as dietary supplements for the management of DM. Further investigation is required to outline which polyol is better for use as a dietary sweetener. Therefore, the present study was aimed at investigating comparatively the antioxidative and antidiabetic effects of xylitol and erythritol in a fructose-fed streptozotocin (STZ)-induced T2D rat model.

4.3 Materials and methods

Kindly refer to Chapter 2; 2.5, 2.6 (sub-sections 2.6.1-2.6.6, 2.6.8-2.6.9) and 2.8 for further details.

4.4 Results

4.4.1 The effects of xylitol and erythritol on mortality, weekly body weight, daily food and fluid intake.

In the present study, a mortality rate of 10.87% (5/46) was observed in the first week of treatment, accounting for 1 animal receiving erythritol at 10% and 4 animals receiving erythritol at 20%. This resulted with a total of 5 animals in the DE10 group and 2 animals in the DE20 group. In **Fig. 4.1 (A)**, the diabetic animals were susceptible to weight loss after the induction of T2D which was further reduced by the treatment of xylitol and erythritol. The weight gain of the animals increased in a gradual manner in the NC group during the entire intervention trial. There was a significantly ($p<0.05$) and dose-dependently lower fluid and food intake observed for xylitol and erythritol-fed groups compared to the DC group, **Fig. 4.1 (B)**, when better results were observed for xylitol compared to erythritol fed groups.

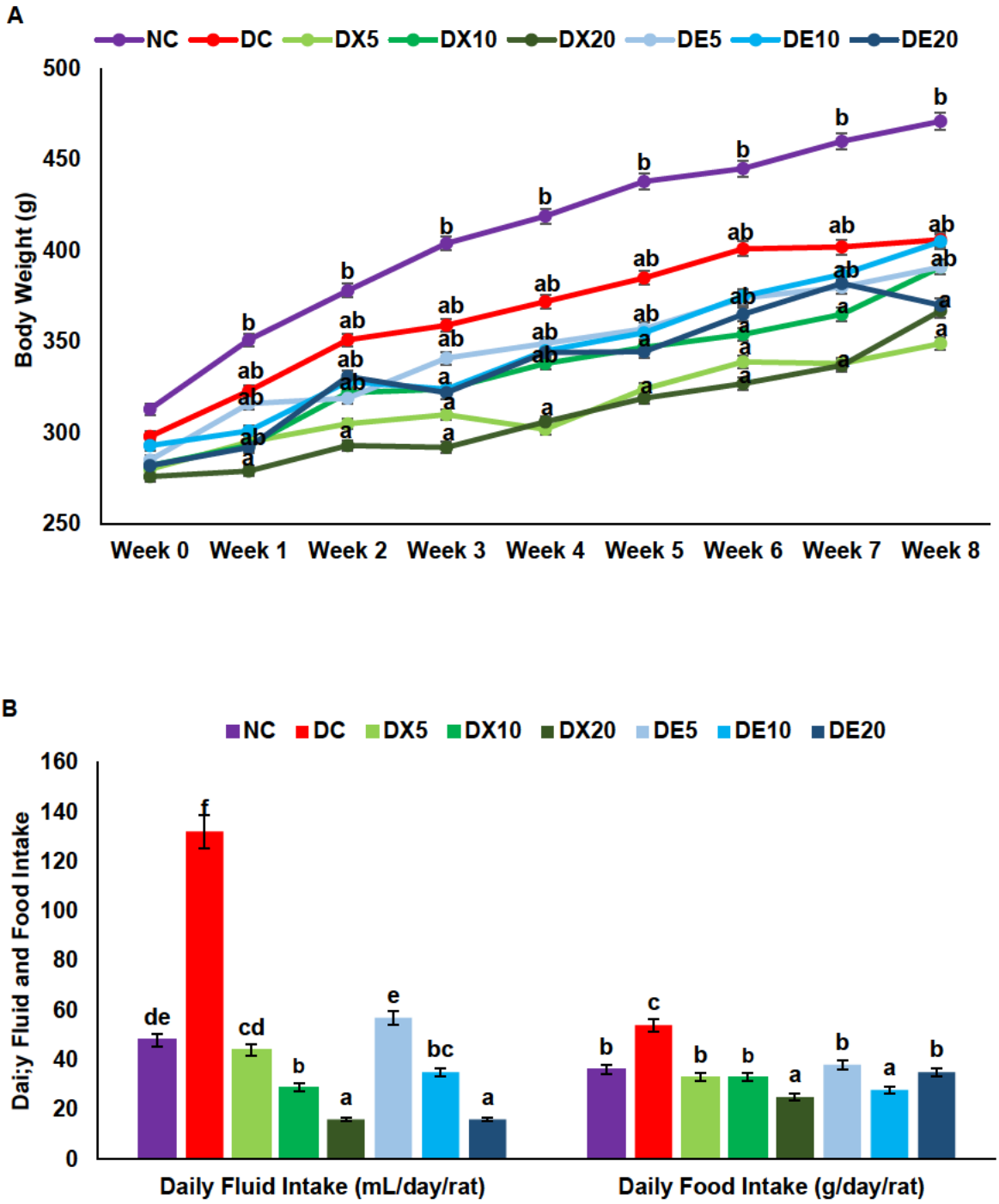

Figure 4.1: (A) Body weight; (B) daily fluid and food intake of pancreas of the experimental groups. Values = mean ± SD; n = 7 (NC), 6 (DX10 and DX20), 5 (DC, DX5 , DE5 and DE10), and 2 (DE 20). [a-f]Different letters presented above the bars for a given group are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA. NC = normal control, DC = diabetic control, DX5 = diabetic xylitol 5%, DX10 = diabetic xylitol 10%, DX20= diabetic xylitol 20%, DE5 = diabetic erythritol 5%, DE10 = diabetic erythritol 10% and DE20 = diabetic erythritol 20%.

4.4.2 The effect of xylitol and erythritol on weekly blood glucose and oral glucose tolerance

In **Fig. 4.2 (A)**, the induction of T2D lead to a significant ($p<0.05$) increase in blood glucose levels in the diabetic groups. A gradual decrease was observed in the experimental groups treated with xylitol and erythritol, with DX10 presenting the highest reduction of 66.67% at the end of the intervention trial when similar results were also observed for DE10 and DE20 groups.

There was an increase in blood glucose levels of all the experimental animals at 30 min after oral dosing with glucose, **Fig. 4.2 (B)**. A significant ($p<0.05$) increase occurred at 30 min for the DC group compared to the NC group. At 120 min, the treatment groups were able to tolerate glucose with DX10, DX20 and DE20 groups showing the better tolerance ability compared to all other groups with no difference with NC group. The total area under the curve (AUC) of DX10, DX 20 and DE20 groups were significantly lower compared to other diabetic groups (DC, DX5, DE5 and DE10), which suggests their better glucose tolerance ability.

4.4.3 The effect of xylitol and erythritol on pancreas weight, relative pancreas weight, serum insulin, HOMA-IR and HOMA-β

The pancreateic weight of xylitol and erythritol-fed groups were higher in the xylitol and erythritol-fed groups when significantly ($p<0.05$) higher weight was observed for DX10 group compared to the DC group, **Table 4.1**.

The serum insulin level and pancreatic β-cell function (HOMA-β) were significantly ($p<0.05$) reduced after the induction of T2D, with concomitant elevation of HOMA-IR as depicted in **Table 4.1**. Treatment with xylitol and erythritol led to a significantly ($p<0.05$) higher serum

insulin level and β-cell function, with improved HOMA-IR scores compared to DC group when best results were observed for DX10 group among all diabetic groups.

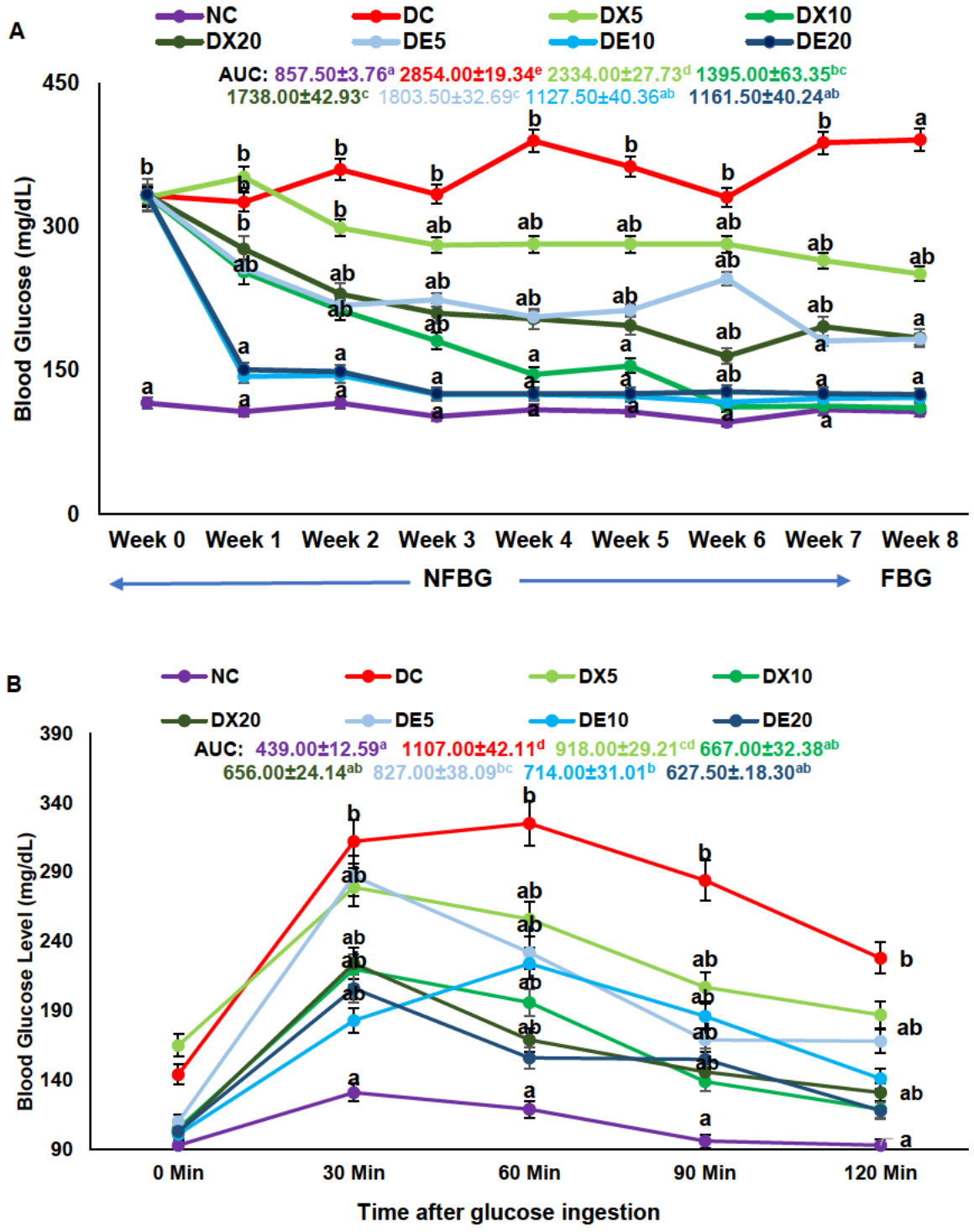

Figure 4.2: **(A) Weekly blood glucose; (B) oral glucose tolerance test (OGTT) and corresponding area under the curve (AUC); in all animal groups at the end of the experimental period**. Values = mean ± SD; n = 7 (NC), 6 (DX10 and DX20), 5 (DC, DX5, DE5 and

DE10) and 2 (DE20).) [a-d]Different letters presented above the lines for a given group are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA. NC = normal control, DC = diabetic control, DX5 = diabetic xylitol 5%, DX10 = diabetic xylitol 10%, DX20= diabetic xylitol 20%, DE5 = diabetic erythritol 5%, DE10 = diabetic erythritol 10% and DE20 = diabetic erythritol 20%.

Table 4.1: Pancreas weight, relative weight, serum insulin level, as well as HOMA-IR and HOMA-β scores of different animal groups at the end of the experimental period.

	Pancreas weight (g)	**Relative pancreas weight (%)**	**Serum insulin (pmol/L)**	**HOMA-IR**	**HOMA - β**
NC	1.75 ± 0.49^{b}	0.37 ± 0.08	45.51 ± 4.99^{b}	1.55 ± 0.17^{a}	90.69 ± 9.94^{c}
DC	1.45 ± 0.11^{a}	0.33 ± 0.06	24.91 ± 2.49^{a}	6.51 ± 0.72^{d}	11.40 ± 1.13^{a}
DX5	1.44 ± 0.38^{a}	0.41 ± 0.07	41.99 ± 7.37^{ab}	3.55 ± 0.86^{c}	27.84 ± 4.90^{b}
DX10	1.60 ± 0.10^{ab}	0.42 ± 0.05	46.99 ± 5.52^{b}	1.66 ± 0.19^{a}	88.72 ± 10.42^{c}
DX20	1.45 ± 0.21^{a}	0.41 ± 0.07	39.88 ± 3.81^{ab}	2.39 ± 0.21^{ac}	38.40 ± 3.66^{b}
DE5	1.45 ± 0.22^{a}	0.37 ± 0.07	36.07 ± 13.12^{ab}	3.11 ± 0.52^{bc}	34.97 ± 12.72^{b}
DE10	1.49 ± 0.18^{a}	0.37 ± 0.04	41.42 ± 9.31^{ab}	1.94 ± 0.39^{ab}	68.31 ± 15.37^{c}
DE20	1.43 ± 0.03^{a}	0.39 ± 0.02	45.55 ± 10.24^{b}	2.31 ± 0.29^{ac}	72.62 ± 16.32^{c}

Results are reported as mean ± SD; n = 7 (NC), 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and 2 (DE20).[a-c]Different superscript letters for a given parameter along a row indicate the significance ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA. NC = normal control, DC = diabetic control, DX5 = diabetic xylitol 5%, DX10 = diabetic xylitol 10%, DX20= diabetic xylitol 20%, DE5 = diabetic erythritol 5%, DE10 = diabetic erythritol 10%, DE20 = diabetic erythritol 20%. HOMA-IR and HOMA-β; Homeostatic model assessment for IR (insulin resistance) and β (β-cell function).

4.4.4 Effect of xylitol and erythritol on serum lipid profile

A significant ($p<0.05$) elevation in the level of total cholesterol, LDL-cholesterol and triglycerides were observed in the DC group when compared to the NC group, **Fig. 4.3**. These parameters were significantly reversed after treatment with xylitol and erythritol, with 10% and 20% doses of xylitol and erythritol showing the most reduction in total cholesterol, LDL-cholesterol and triglycerides concentrations. There was no significant difference observed for the HDL-cholesterol among all the experimental groups.

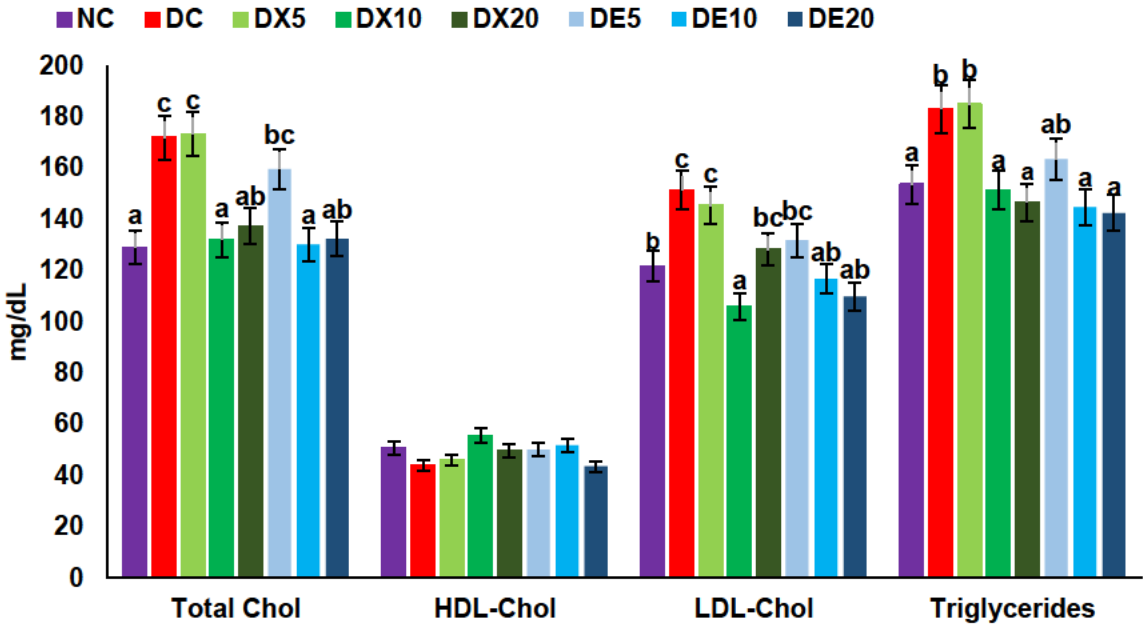

Figure 4.3: **Serum lipid profile of all animal groups at the end of the experimental period**. Values = mean ± SD; n = 7 (NC), 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and 2 (DE20).) [a-d]Different letters presented above the bars for a given group are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA. NC = normal control, DC = diabetic control, DX5 = diabetic xylitol 5%, DX10 = diabetic xylitol 10% DX20= diabetic xylitol 20%, DE5 = diabetic erythritol 5%, DE10 = diabetic erythritol 10% and DE20 = diabetic erythritol 20%.

4.4.5 Effect on organ function related biomarkers

Induction of T2D led to increased levels of ALT, AST, CK-MB and Creatinine in the diacetic control group, **Table 4.2**. Treatment with xylitol and erythritol significantly ($p<0.05$) reduced the levels of these parameters. DX10 group had the lowest levels for ALT, AST and CK-MB, while DE10 had the lowest level for creatinine. There was no significant difference in urea and uric acid among all the experimental groups.

Table 4.2: Cocentration of organ function related biomarkers in the serum of all experimental groups at the end of the experimental period.

Parameters/ Groups	ALT U/L	AST U/L	CK-MB U/L	Creatinine mg/dL	Urea mg/dL	Uric acid mg/dL
NC	83.17±1.47[a]	56.33±6.66[a]	421.67±120.67[ab]	1.61±0.04[ab]	23.33±12.74	2.44±0.82
DC	89.25±2.98[b]	167.50±63.92[b]	536.35±141.52[b]	1.98±0.12[b]	33.33±8.62	2.86±0.62
DX 5	84.40±2.07[a]	90.20±69.52[ab]	547.50±58.46[b]	1.57±0.13[ab]	26.67±7.23	2.91±0.75
DX 10	83.67±1.03[a]	69.40±20.00[a]	304.00±12.73[a]	1.60±0.18[ab]	26.75±4.03	3.29±0.11
DX 20	84.00±1.67[a]	88.50±20.52[ab]	375.97±68.20[ab]	1.82±0.24[ab]	23.67±14.19	2.61±0.61
DE 5	84.20±1.64[a]	70.33±13.69[a]	493.20±94.73[ab]	1.73±0.29[ab]	34.00±6.24	3.09±0.32
DE 10	84.50±1.91[a]	78.50±4.94[a]	344.98±66.23[ab]	1.45±0.17[a]	40.00±2.00	2.31±0.54
DE 20	84.50±0.71[a]	72.50±4.94[a]	308.50±45.27[a]	1.82±13.00[ab]	38.00±4.24	2.61±0.16

Values = mean ± SD; n = 7 (NC), 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and 2 (DE20).).[a-b]Different superscript letters for a given parameter along a row indicate the significance ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA. NC = normal control, DC = diabetic control, DX5 = diabetic xylitol 5%, DX10 = diabetic xylitol 10%, DX20= diabetic xylitol 20%, DE5 = diabetic erythritol 5%, DE10 = diabetic erythritol 10%, DE20 = diabetic erythritol 20%.

4.4.6 Effects of xylitol and erythritol on oxidative biomarkers

In **Fig. 4.4**, significantly ($p<0.05$) depleted GSH level was observed for the DC group in both the pancreas and serum. In the presence of xylitol and erythritol, the GSH levels were reversed. Although 10 and 20% groups of xylitol and erythritol showed higher GSH concentrations compared to DC group, the highest level was observed in the DX10 group compared to all other groups, both in pancreatic tissue and serum.

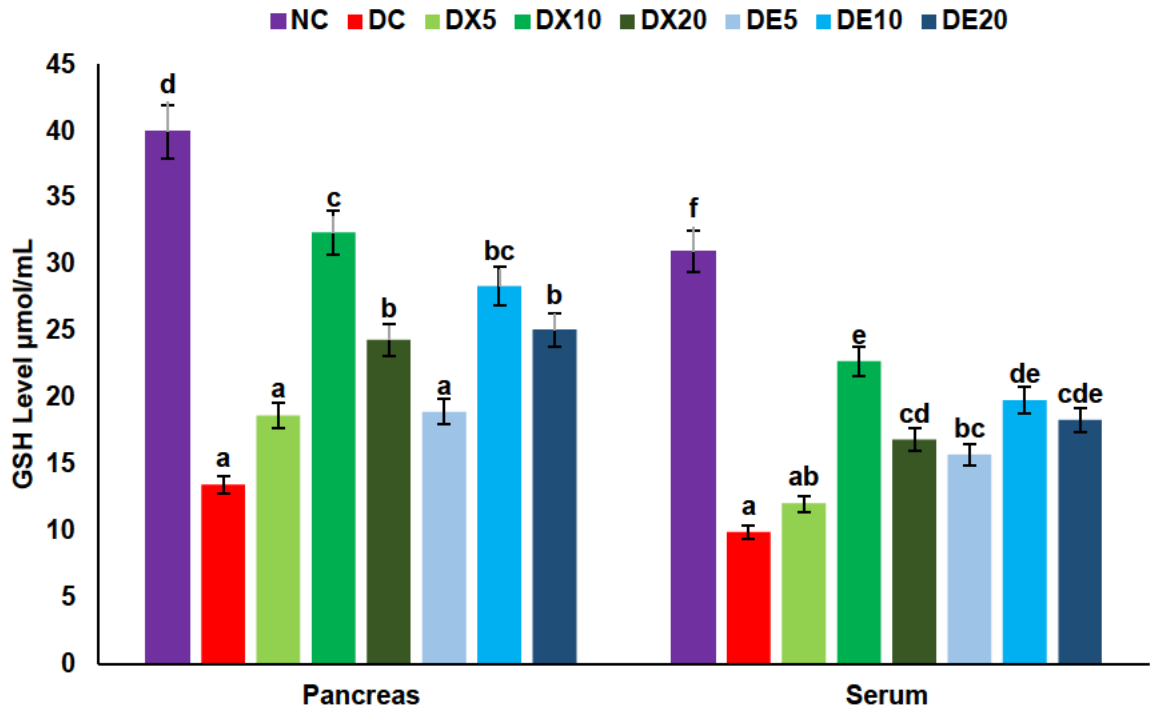

Figure 4.4: **Reduced glutathione (GSH) levels in the pancreas and serum of all animal groups at the end of experimental period**. Values = mean ± SD; n = 7 (NC), 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and 2 (DE20).) [a-f]Different letters presented above the bars for a given group are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA. NC = normal control, DC = diabetic control, DX5 = diabetic xylitol 5%, DX10 = diabetic xylitol 10% DX20= diabetic xylitol 20%, DE5 = diabetic erythritol 5%, DE10 = diabetic erythritol 10% and DE20 = diabetic erythritol 20%.

Fig. 4.5 represents the MDA level in the pancreas and serum of the experimental groups. Induction of T2D led to a significant ($p<0.05$) upsurge in MDA level of the DC group compared to the NC group. The treatment groups were able to reduce the MDA level in both the pancreas and serum. A significant ($p<0.05$) reduction was observed in the pancreas and serum of DX10 and DX20 groups and in the serum of DE10 and DE20 groups compared to t the DC group.

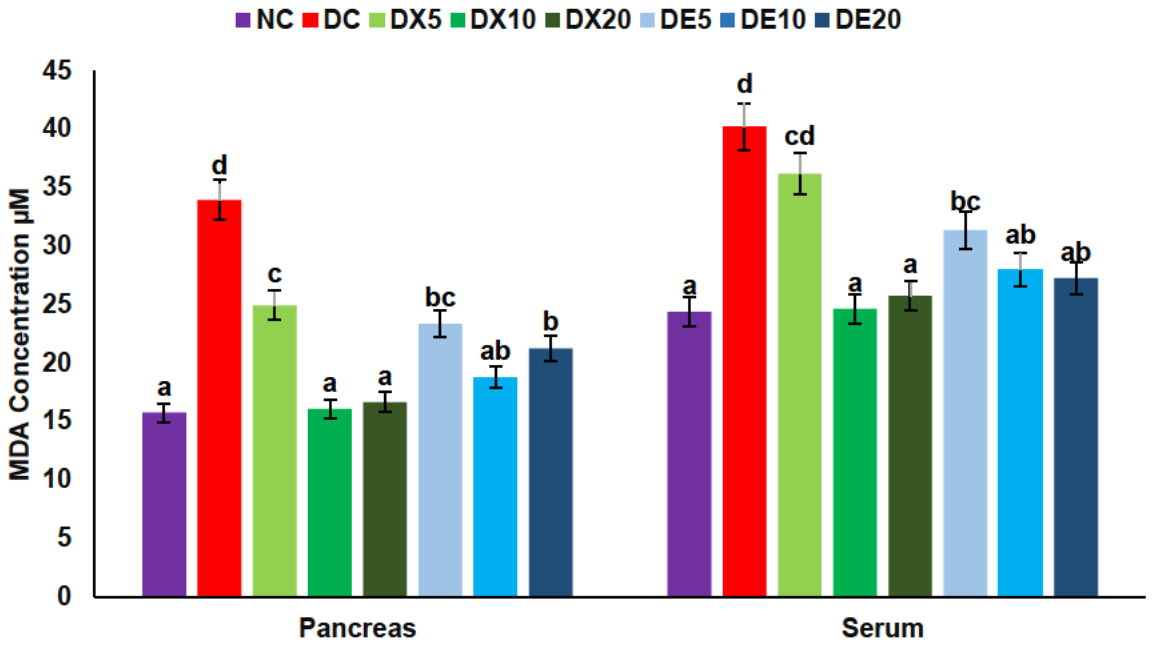

Figure 4.5: **Thiobarbituric acid reactive substances (MDA equivalent) in the pancreas and serum**. Values = mean ± SD; n = 7 (NC), 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and 2 (DE20).) [a-e]Different letters presented above the bars for a given group are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA. NC = normal control, DC = diabetic control, DX5 = diabetic xylitol 5%, DX10 = diabetic xylitol 10% DX20= diabetic xylitol 20%, DE5 = diabetic erythritol 5%, DE10 = diabetic erythritol 10% and DE20 = diabetic erythritol 20%.

Superoxide dismutase activity was significantly ($p<0.05$) reduced for the diabetic control group when compared to the normal control group in both the pancreas and serum, **Fig. 4.6**. The enzyme activity increased after treatment with xylitol and erythritol, with DX10 group showing a significant ($p<0.05$) increase in both the pancreas and serum compared to DC group.

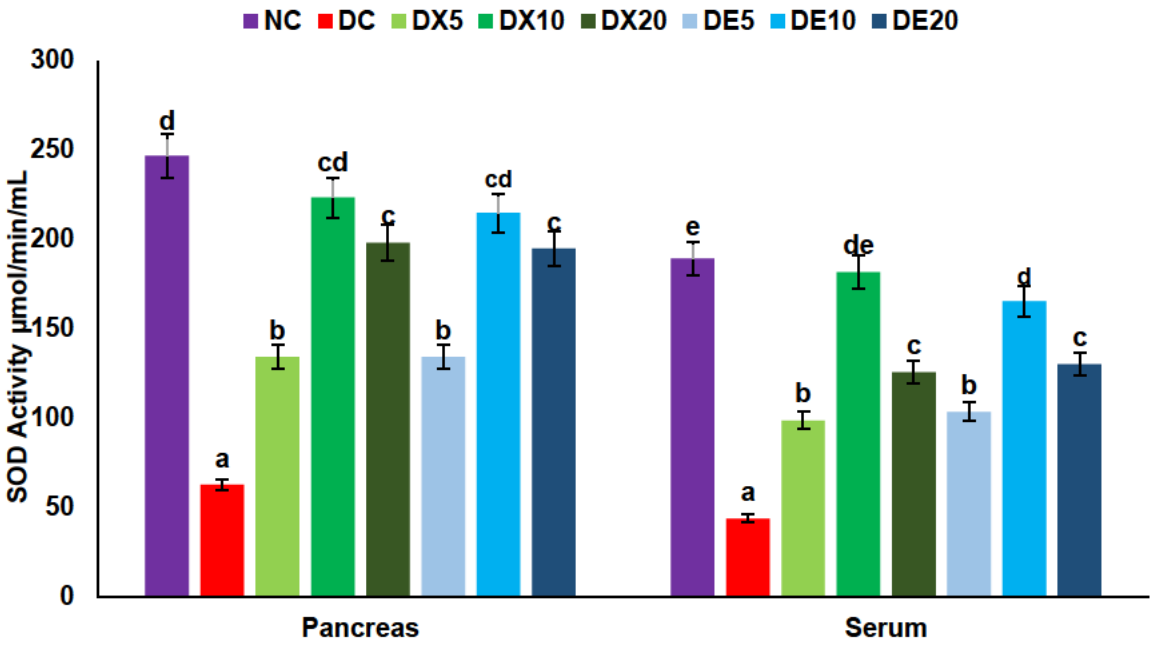

Figure 4.6: **Superoxide dismutase activity in the pancreas and serum of the experimental groups**. Values = mean ± SD; n = 7 (NC), 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and 2 (DE20).) [a-e]Different letters presented above the bars for a given group are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA. NC = normal control, DC = diabetic control, DX5 = diabetic xylitol 5%, DX10 = diabetic xylitol 10% DX20= diabetic xylitol 20%, DE5 = diabetic erythritol 5%, DE10 = diabetic erythritol 10% and DE20 = diabetic erythritol 20%.

In **Figure 4.7**, a significant ($p<0.05$) depletion of catalase activity was observed in the DC group. Treatment with xylitol and erythritol were able to increase the activity, with DX10 showing the most significant ($p<0.05$) effect when compared to the other diabetic treatment groups.

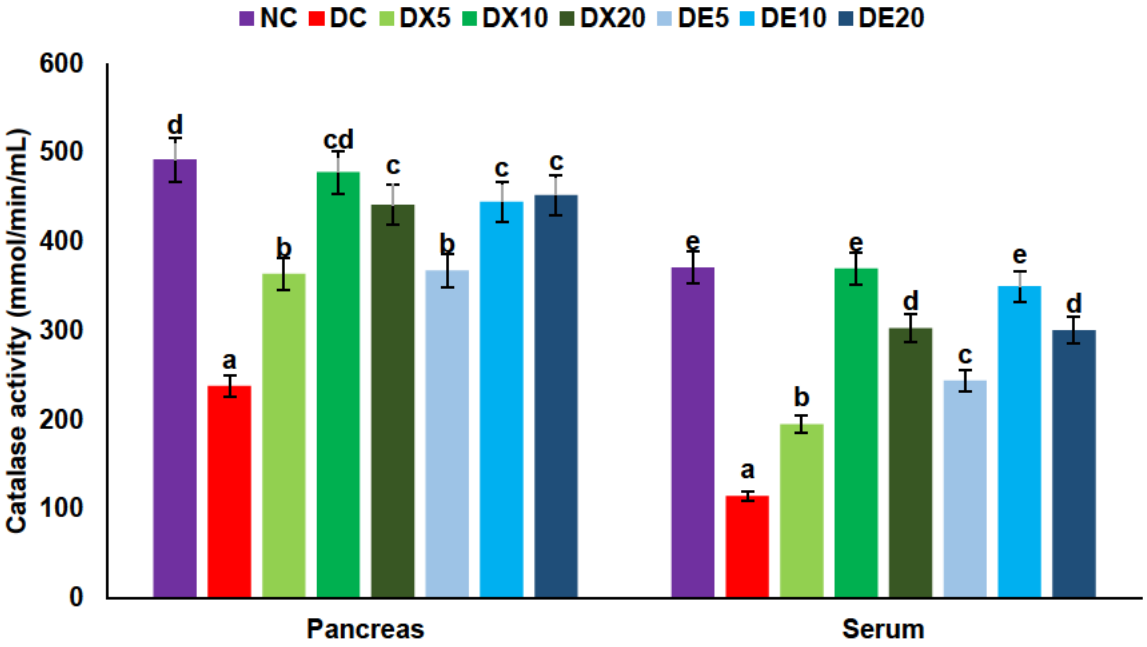

Figure 4.7: **Catalase activity in the pancreas and serum of the experimental groups**. Values = mean ± SD; n = 7 (NC), 6 (DX10 and DX20), 5 (DC, DX5 , DE5 and DE10) and 2 (DE20). [a-d]Different letters presented above the bars for a given group are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA. NC = normal control, DC = diabetic control, DX5 = diabetic xylitol 5%, DX10 = diabetic xylitol 10% DX20= diabetic xylitol 20%, DE5 = diabetic erythritol 5%, DE10 = diabetic erythritol 10% and DE20 = diabetic erythritol 20%.

4.4.7 Effects of xylitol and erythritol on the histological analysis of the pancreas.

The morphological changes of the pancreatic tissues of the experimental groups are depicted in **Fig. 4.8**. The NC group showed intact pancreatic islet and acinar cells. The DC group presented a significantly smaller islet compared to the NC group. The morphology was significantly restored for the xylitol and erythritol treatment groups, DX10 and DX20 groups showing the better results compared to all other treatment groups.

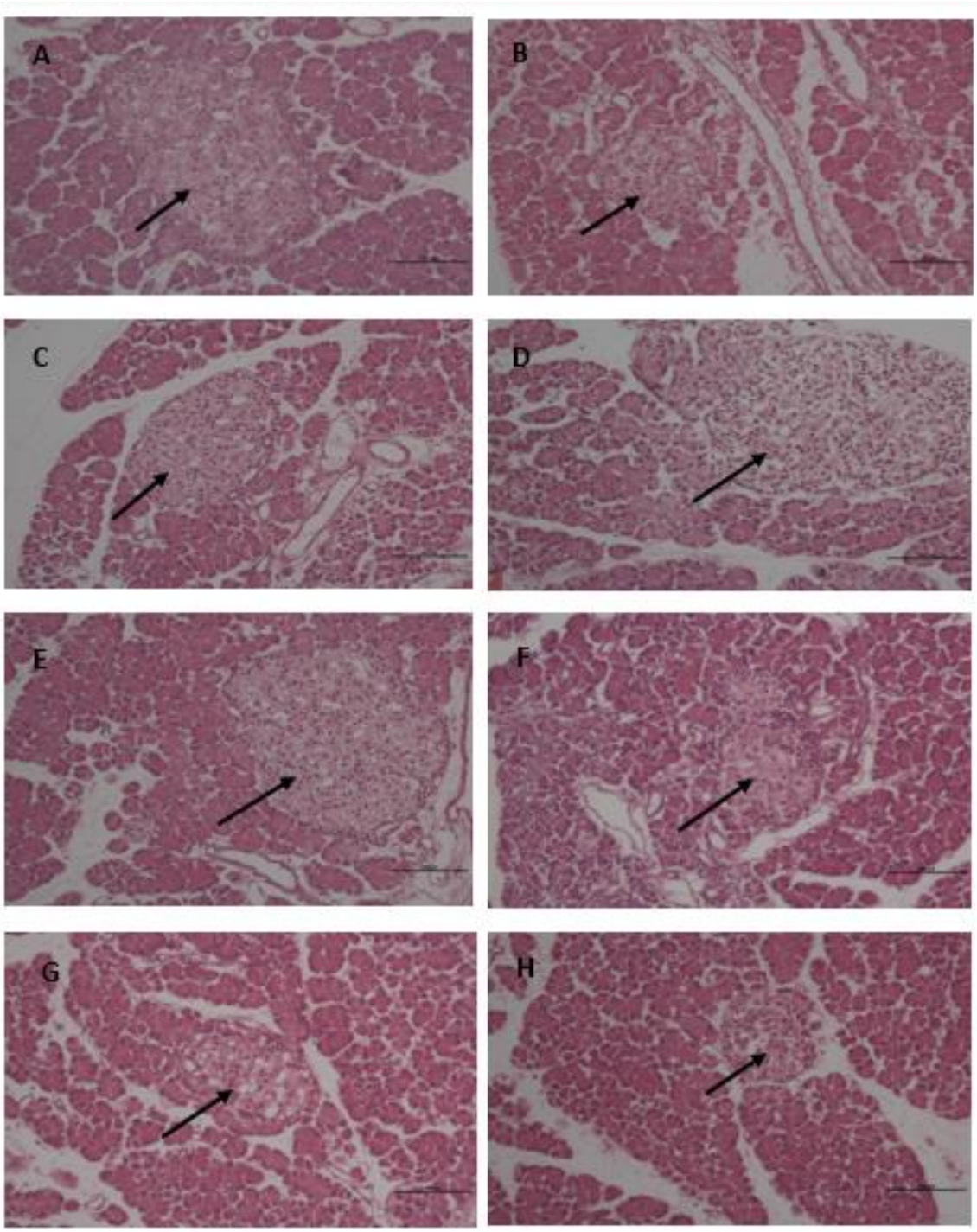

Figure 4.8: **Morphological changes in pancreatic tissues of experimental groups**. Magnification: 200x. (A) = NC, (B) = DC, (C) = DX5, (D) = DX10, (E) = DX20, (F) = DE5, G = DE10 and H = DE20. NC = Normal control, DC = Diabetic control, DX 5 = Diabetic xylitol 5%, DX 10 = Diabetic xylitol 10%, DX 20= Diabetic xylitol 20%, DE 5 = Diabetic erythritol 5%, DE 10 = Diabetic erythritol 10% and DE 20 = Diabetic erythritol 20%. Arrow: β-cells

4.5 Discussion

Xylitol and erythritol are widely used as sugar substitutes because of their diverse beneficial effects on health (Wölnerhanssen *et al.*, 2019). Their lower caloric values, insulinemic response and glycemic index have made them acceptable for use by diabetic individuals (Wolever *et al.*, 2002). Previous studies have reported the hypoglycemic and antidiabetic effects of these sugar alcohols (Islam, 2011; Kishore *et al.*, 2012 ; Chukwuma *et al.*, 2018). However, it is not known which one of these sugar alcohols is most effective in ameliorating diabetes-related parameters. The present study investigated to comparatively examine the antioxidative and antidiabetic effects of xylitol and erythritol using a fructose-fed streptozotocin (STZ)-induced T2D rat model.

In the present study, animals in diabetic groups were fed with 10% fructose solution ad libitum instead of drinking water to induce insulin resistance, one of the two major pathogenesis of T2D. Previous animal and human studies have associated the intake of fructose with weight gain (Rizkalla *et al.*, 1993; Stanhope *et al.*, 2009; Moraes-Silva *et al.*, 2013). The consumption of fructose can alter the production and secretion of appetite regulating hormones and peptide which include ghrelin, leptin and insulin (Teff *et al.*, 2004; Lindqvist *et al*., 2008). Animal studies have reported the development of leptin resistance after fructose consumption which was linked to heightened sensation of hunger and weight gain (Huang *et al.*, 2004; Shapiro *et al.*, 2008). However, although 2 weeks feeding of fructose solution increased the food and fluid intake and body weight (data not shown) as well as insulin resistance (HOMA-IR) (Table 4.1) in diabetic control group, xylitol and erythritol feeding dose-dependently and significantly reversed these parameters **(Fig. 4.1 and Table 4.1)**. Several studies have reported that consumption of sugar alcohols can delay gastric emptying, reduce food intake, fluid intake and body weight gain (Amo *et al.*, 2011; Islam, 2011; Chukwuma and Islam, 2017a). Additionally, polydipsia is a commonly known

sign for DM which was observed in the diabetic control group (**Fig. 4.1B**). Treatment with xylitol and erythritol reduced fluid intake significantly ($p<0.05$) compared to the DC group. The ability of xylitol and erythritol to supress polyphagia, polydipsia as well as lowering body weight gain indicate their potential beneficial effects in treating and managing diabetes.

The aetiology of T2D is characterized by peripheral insulin resistance, impaired regulation of hepatic glucose production, and deteriorating pancreatic β-cell function (Kaku, 2010). This is consistent with the pancreatic weight loss (**Table 4.1**), elevated blood glucose level (**Fig. 4.2A**), depleted β-cell function and serum insulin level (**Table 4.1**) of the diabetic control group. This is attributed by the decreased pancreatic islet size and depleted pancreatic β-cells (**Fig. 4.8A**), with β-cell dysfunction and ultimately insulin secretion was impaired and elevated glucose prevails manifesting into hyperglycemia resulting in T2D (Galicia-Garcia *et al.*, 2020). Xylitol and erythritol treatments were able to reduce blood glucose level (**Fig. 4.2A**), with DX10, DE10 and DE20 groups presenting the higher reductions compared to all other treatment groups. According to the pattern of glucose reduction **(Fig. 4.2A)**, although drastic reduction was observed in the DE10 and DE20 groups, the reduction in DX10 group was progressive and ultimately reach into the normal level after 8 weeks treatment. Considering this, 10% xylitol group may be a better option to reduce the blood glucose level in a physiologically safer way compared to similar or higher dose of erythritol to avoid drastic hypoglycemia. Drastic reduction of blood glucose levels might also be a reason for the death of some animals in DE10 and DE20 groups. Considering this, xylitol may be a preferred option for the reduction of hyperglycaemia compared to erythritol. However, further studies are required in order to identify the most effective safer dose of erythritol for the management of hyperglycaemia.

Additionally, elevated pancreatic weight, β-cell number, β-cell function and serum insulin level were observed in the xylitol and erythritol treated groups (**Fig. 4.8 and Table 4.1**),

when the effect of 10% xylitol was better than the similar or higher dose of erythritol. The ability of xylitol and erythritol to reduce the blood glucose level, elevate serum insulin level, improve pancreatic morphology and β-cell dysfunction ascertain their antidiabetic potentials, when the effects of 10% xylitol was more pronounced compared to similar or higher dose of erythritol, particularly in terms of pancreatic weight, β-cell function, insulin secretion and pancreatic islets morphology **(Fig. 4.8 and Table 4.1)**. Additionally, although 10% and 20% xylitol and erythritol were most effective in improving glucose tolerance ability (**Fig. 4.2 B**) compared to the other diabetic treatment groups, the effects of 10% xylitol showed superior results compared to all other treatment groups of xylitol and erythritol. The results of this study is also supported by the results of a previously published study where 10% xylitol showed the better antidiabetic potential in terms of reducing blood glucose level, stimulating insulin secretion and improving pancreatic morphology (Rahman and Islam, 2014). Considering this, xylitol may be preferred as an antidiabetic sweetener compared to erythritol.

Dyslipidemia is a group of lipoprotein abnormalities characterized by the increased levels of total cholesterol, LDL-cholesterol, triglycerides, and decreased HDL-cholesterol level (Jialal and Singh, 2019). This was evident in the diabetic control group (**Fig. 4.3**), which indicates an occurrence of diabetic dyslipidaemia. The effects of xylitol and erythritol on dyslipidemia and diabetic dyslipidemia have been reported in previously published studies (Rahman and Islam, 2014; Kawano *et al.*, 2021) .Treatment with the higher doses of xylitol and erythritol reversed these parameters except HDL-cholesterol, when the effects of 10% and 20% xylitol and erythritol were quite similar and best and similar effect in lowering LDL-cholesterol was observed in 10% xylitol and 20% erythritol fed groups. Although the reduction of triglyceride was also highest in the 20% erythritol fed group, the associated hypoglycemia as well as the death of animals in this group raised a concern about the safety of the 20% dose of erythritol.

These data again proved the effects of xylitol and erythritol in the reduction of diabetic dyslipidaemia, when xylitol may be a better choice considering the toxicity of the higher doses of erythritol.

The multisystemic nature of T2D implies that complications and comorbidities can affect numerous organ systems (Forbes and Cooper, 2013). The elevation of ALT, AST, CK-MB, creatinine, urea and uric acid have been reported in diabetic complications (Fazel *et al.*, 2005; Amartey *et al*., 2015; Arika *et al.*, 2016), which is consistent with the elevated levels in the diabetic control group in our study (**Table 4.1**). These elevations may be due to damage in the heart, kidney and liver tissues (Fazel *et al.*, 2005; Amartey *et al*., 2015; Arika *et al.*, 2016), Although the treatment with xylitol and erythritol was able to reverse most of these biomarkers, the higher level of serum urea observed in the 10% and 20% erythritol fed groups again raised the concern about the safety of the higher doses of erythritol, when consistently better effects were observed for all doses of xylitol. This observation indicates the protective effect of xylitol against diabetes complications, when further studies are needed in order ascertain the efficacy and safety of erythritol.

It is well established that oxidative stress plays a major role in the onset and development of T2D (Chikezie *et al*., 2015; Oguntibeju, 2019). It is defined as an imbalance between the generation and elimination of reactive oxygen species in favour of the formation of oxidants (Pizzino *et al.*, 2017). This redox imbalance is a consequence of various abnormalities, including hyperglycemia, insulin resistance, hyperinsulinemia, and dyslipidaemia (Giacco and Brownlee, 2010; Asmat *et al*., 2016). In **Fig. 4.4 – 4.7**, the reduced levels of GSH, SOD and CAT activities, with increased level of MDA in both the pancreatic tissue and serum of the diabetic control group is an indication of oxidative damage. Xylitol and erythritol were able to reverse the activities and levels of these biomarkers in both the pancreatic tissue and serum. However, the 10% xylitol treatment showed better efficacy in ameliorating oxidative

stress compared to all other doses of xylitol and erythritol, which could be attributed by the improved morphology of the pancreas (**Figure 4.8 D**) and β-cell function (**Figure 4.2 C**). It has also been reported that the oxygen radical scavenging capacity of sugar alcohols is dependent on the number of their aliphatic hydroxyl group (Kang *et al.*, 2007), which is higher in xylitol compared to erythritol.

4.6 Conclusion

The data of this study suggest that xylitol and erythritol have therapeutic potential against T2D and oxidative stress. However, it was evident that 10% xylitol had better therapeutic efficacy compared to similar or higher doses of xylitol and erythritol used in this study, which was proven by its ability to supress hyperglycemia, improve glucose tolerance, insulin secretion, pancreatic morphology and β-cell dysfunction, attenuation of dyslipidemia and oxidative stress. On the other hand, although 10% and 20% of erythritol showed comparable effects compared to 10% xylitol, associated sudden hypoglycaemia as well as mortality and higher serum urea levels in erythritol fed groups raised concerns about its safety. Considering all above, xylitol can be preferred antidiabetic sweetener over erythritol when 10% xylitol has been found the most effective dose compared to all other doses of xylitol and erythritol used in this study. Further studies are needed in order to ascertain the most effective safer dietary dose of erythritol for the management of T2D and its associated complications.

CHAPTER 5

Comparative effects of xylitol and erythritol in improving antioxidant, purinergic and cholinergic dysfunction, and lipid metabolic homeostasis in hepatic injury in type 2 diabetic rats

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Preface: This article investigated the comparative therapeutic effect of xylitol and erythritol on redox imbalance, purinergic and cholinergic dysfunction, and lipid dysmetabolism in hepatic tissue of fructose-streptozotocin (STZ) induced T2D rats. This article is currently under preparation for submission to an international peer-reviewed journal.

5.1 Abstract

The present study investigated the comparative therapeutic effect of xylitol and erythritol on glycogen content, redox imbalance, purinergic and cholinergic dysfunction, and lipid dysmetabolism in hepatic tissue of diabetic rats. Seven weeks old male Sprague-Dawley rats were divided into eight groups as follows: Normal control (NC), Diabetic control (DC), Diabetic xylitol 5% (DX5), Diabetic xylitol 10% (DX10), Diabetic xylitol 20% (DX20), Diabetic erythritol 5% (DE5), Diabetic erythritol 10% (DE10) and Diabetic erythritol 20% (DE20). Type 2 diabetes was induced in the diabetic groups and after confirmation of diabetes, the xylitol and erythritol groups were supplied with their respective solutions. The animals were humanely sacrificed after 8 weeks of treatment and their hepatic tissues were harvested. Treatment with 10% xylitol compared to the other treatment groups had significantly higher GSH level, relatively lower MDA level, ATPase and acetylcholinesterase activities, with relatively higher SOD, catalase and ENTPDase activities. It further modulated lipid metabolism and restored hepatic morphology. The data suggests that xylitol at 10% had better therapeutic effect against T2D and its complications. However, further clinical studies are still required to affirm these findings.

Keywords: Xylitol, Erythritol, Type 2 Diabetes, Oxidative stress, Hepatotoxicity

5.2 Introduction

Type 2 diabetes (T2D) has become a tremendous health problem globally, as it accounts for over 90% of all diabetic cases (IDF, 2019b). It is characterised by relative insulin deficiency instigated by pancreatic β-cell dysfunction and insulin resistance in target organs (ADA, 2019). T2D increases the risk of several other diseases triggered by macrovascular and microvascular damage, and it has negative impacts on several organs including but not limited the kidney, heart, brain and liver (Ahmed *et al.*, 2010; Berbudi *et al.*, 2020). Non-alcoholic fatty liver disease (NAFLD), liver cirrhosis and hepatocellular carcinoma are part of liver diseases associated with T2D and are considered as a significant contributor to mortality in T2D (Garcia-Compean *et al.*, 2009; Islam *et al.*, 2020).

The liver is a vital organ in the regulation of metabolic homeostasis and adaptation of nutrients availability and deprivation (Loria *et al*.., 2008; Bechmann *et al.*, 2012; Szablewski, 2017). During the inception of diabetes, major biochemical and functional abnormalities occur in the liver, such as alterations in carbohydrate, lipid and protein metabolism and fluctuations in antioxidant status (Ding *et al.*, 2018). The generation of free radicals from hyperglycaemia-induced oxidative stress leads to liver damage triggered by cellular necrosis and inflammation (Hickman and Macdonald, 2007; Guicciardi *et al.*, 2013).

Additionally, excessive consumption of added sugars in food has been in the forefront of liver disease and the metabolic syndrome (Abdelmalek and Day, 2015; Vreman *et al.*, 2017). Numerous studies have reported that fructose in particular can induce liver failure by stimulating de novo lipogenesis and blocking β-oxidation of fatty acids (Jensen , *et al.*, 2018; Berná and Romero-Gomez, 2020). Since it is well known that dietary modification is used as a therapeutic role in the management of liver disease, therefore, the use of alternative sugars could be possible in modulating hepatic failure (Bischoff *et al.*, 2020; Kakleas *et al*., 2020).

Sugar alcohols have a different level of sweetness compared to sugar and are known for their effects on blood glucose response compared to table sugar; hence, used as sweeteners or sugar alternatives (Livesey, 2003; Grembecka, 2015). Numerous studies have reported that xylitol and erythritol have potential beneficial effects in the management and prevention of obesity, diabetes and other related metabolic disorders (Wölnerhanssen *et al.*, 2019)(Msomi *et al*., 2021). In a previous study, Amo et al. (2011) observed that xylitol-fed rats had significantly lower visceral fat mass and plasma lipid concentrations, with an increase in lipogenic enzymes, fatty acid oxidation and ChREBP compared to high-fat diet-fed rats (Amo *et al.*, 2011). The supplementation of 10% xylitol has demonstrated the ability to improve diabetes associated parameters including reduction in blood glucose and serum fructosamine levels, better glucose tolerance in diabetic rats (Islam, 2011; Rahman and Islam, 2014). Mushtaq et al. (2014), reported the ability of xylitol as a supplement for three weeks to reduce serum glucose in normal and diabetic rats with a reduction in food intake and weight gain in a dose dependent manner (Mushtaq *et al.*, 2014). On the other hand, erythritol has been reported to reduce oxidative stress markers such as lipid peroxidation and protein glycosylation in diabetic rats (Yokozawa *et al*., 2002). In another study, the supplementation of erythritol in streptozotocin-induced diabetic rats displayed an endothelium-protective effect (den Hartog *et al.*, 2010). In another previous study, the mean levels of plasma glucose and insulin in healthy human subjects remained unaltered up to 3 h after a single oral dose of erythritol at a dose of 1 g/kg body weight (Bornet *et al.*, 1996b).

In the above-mentioned studies, xylitol and erythritol have been extensively studied in terms of their antidiabetic properties, there is still a dearth of literature on their comparative therapeutic effect against T2D and its complications. Therefore, the present study was conducted to examine the comparative therapeutic effect of xylitol and erythritol on redox

imbalance, purinergic and cholinergic dysfunction, and lipid dysmetabolism in hepatic tissue of fructose-fed streptozotocin (STZ)-induced T2D rats.

5.3 Materials and methods

Kindly refer to Chapter 2; 2.6 (sub sections 2.6.1-2.6.3, 2.6.5-2.6.12) and 2.8 for further details.

5.4. Results

Table 5.1: The effects of xylitol and erythritol on liver weight, relative liver weight and liver glycogen levels in the different animal groups at the end of the intervention period.

	Parameters		
	Liver weights (g)	**Rel. liver weights (%)**	**Liver glycogen (µg/mg)**
NC	13.23±1.67	2.80±0.18^{a}	28.27±4.37^{d}
DC	10.96±1.14	3.19±0.13^{b}	14.61±0.43^{a}
DX5	11.78±1.09	3.42±0.38^{b}	16.63±1.09^{ab}
DX10	12.66±1.04	3.02±0.07^{ab}	23.05±1.06^{c}
DX20	12.19±1.51	3.42±0.65^{b}	17.18±0.27^{ab}
DE5	12.41±1.05	3.19±0.32^{b}	13.66±1.12^{a}
DE10	12.63±1.38	3.12±0.19^{b}	21.41±1.46^{bc}
DE20	12.25±0.08	3.32±0.14^{b}	16.42±0.79^{ab}

Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). $^{a-d}$Different superscript letters within a column for a given parameter indicate the significance of difference ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 = Diabetic erythritol 5%, DE10 = Diabetic erythritol 10% and DE20 = Diabetic erythritol 20%.

The NC and treatment groups had higher liver weights compared to the DC group, however, the data were not significantly different. The relative liver weight of the normal group (NC) was significantly ($p < 0.05$) lower than that of most of the diabetic groups, when the

treatment of xylitol or erythritol were not significantly effective to reduce the relative lever weight. However, the treatment group DX10 showed lower relative weight compared to other treatment groups with no significant difference with NC group. Additionally, the induction of diabetes significantly decreased the level of liver glycogen compared to NC group. The treatment with xylitol and erythritol improved the liver glycogen levels when only significantly so for 10% xylitol and 10% erythritol fed groups and best improvement was observed for 10% xylitol fed group **(Table 5.1)**.

The reduced glutathione (GSH) concentration of the liver of all the groups is presented on **Fig. 5.1**. The induction of T2D in the DC group resulted in significant ($p<0.05$) depletion of GSH level when compared to the normal control (NC) group. Treatment with xylitol and erythritol significantly ($p<0.05$) increased the level of GSH, with the DX10 group presenting the highest increase compared to all other treated groups.

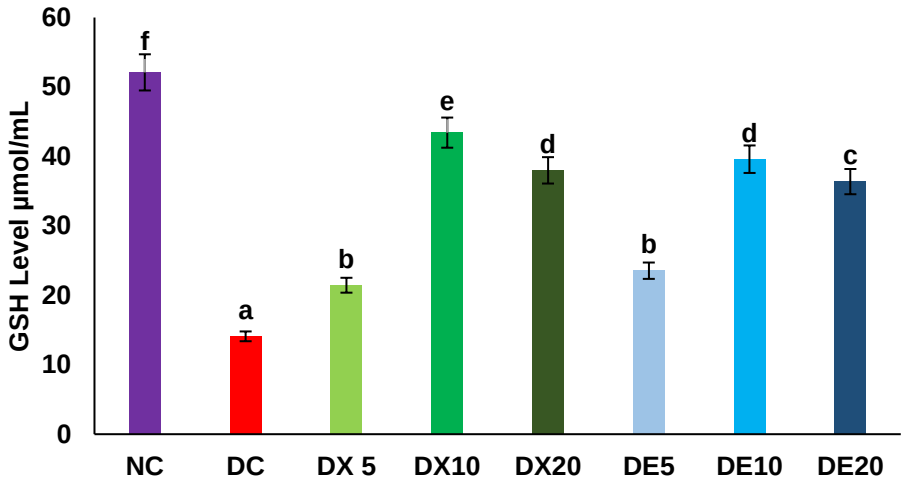

Figure 5.1: **Effects of Xylitol and Erythritol on GSH level in the liver at the end of the intervention trial**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE 20). [a-f]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

The liver lipid peroxidation of the experimental groups is shown in **Fig. 5.2**. The DC group presented significantly ($p<0.05$) elevated hepatic MDA level after the induction of diabetes. The MDA level was significantly ($p<0.05$) reduced in all animal groups treated with xylitol or erythritol when higher reductions were observed in the DX10 and DX20 groups compared to all other treatment groups except DE10 group.

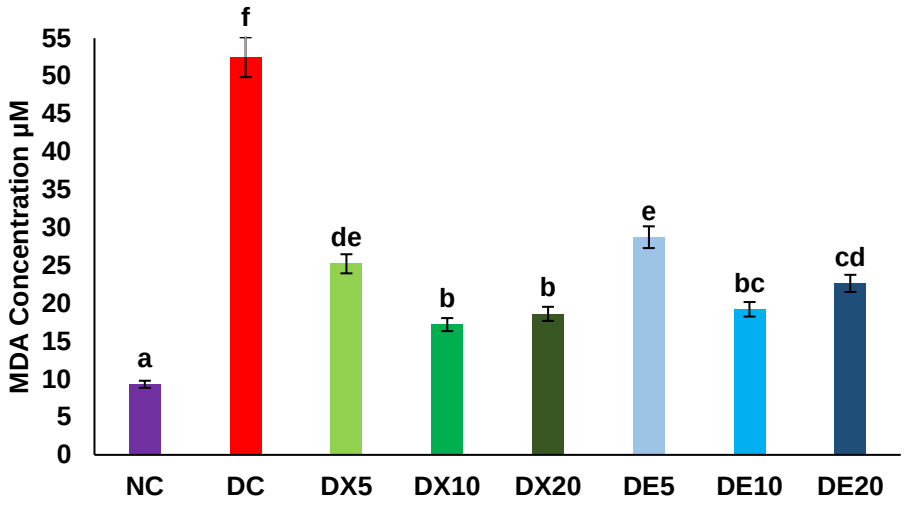

Figure 5.2: **Effect of Xylitol and Erythritol on MDA level in the liver at the end of the intervention trial**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE 20). [a-f]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

The activity of superoxide dismutase (SOD) in the liver of all the animal groups is shown in **Fig. 5.3**. The SOD activity of the DC group was significantly ($p < 0.05$) decreased compared to the normal control group after the induction of diabetes. Treatment with xylitol and erythritol significantly ($p<0.05$) elevated the activity of SOD, in particular the DX10 and

DX20 groups. Significantly higher SOD activities were also observed in the other treated groups compared to the DC group.

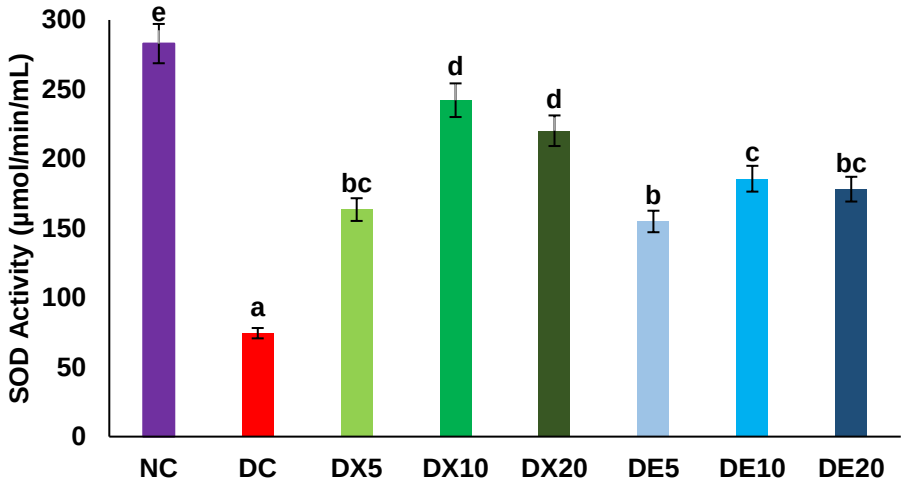

Figure 5.3: **Effect of Xylitol and Erythritol on SOD activity in the liver at the end of the intervention trial**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE 20). [a-e]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

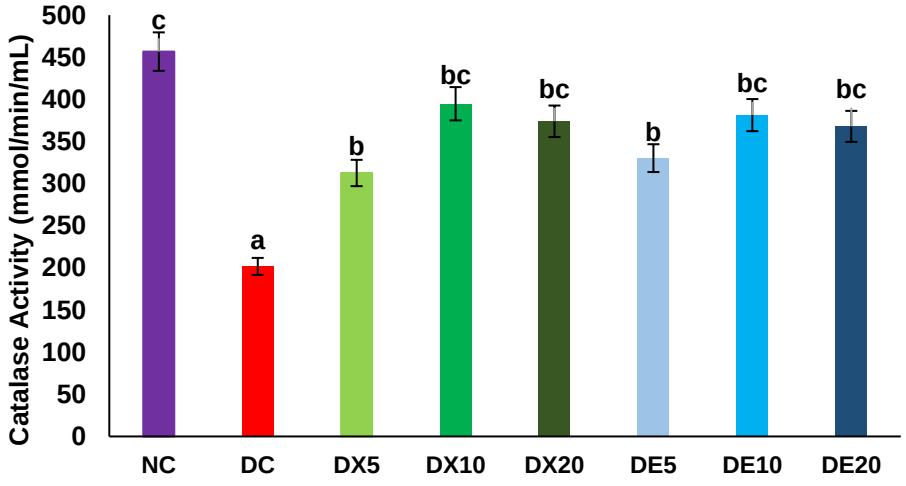

Figure 5.4: **Effect of Xylitol and Erythritol on catalase activity in the liver at the end of the intervention trial**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and

DE10) and n=2 (DE 20). [a-c]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

Fig. 5.4 shows the data for catalase activity of the experimental groups at the end of the intervention trial. The DC group presented significantly ($p<0.05$) decreased catalase activity compared to the NC group. Treatment was xylitol and erythritol was able to significantly reverse the activity.

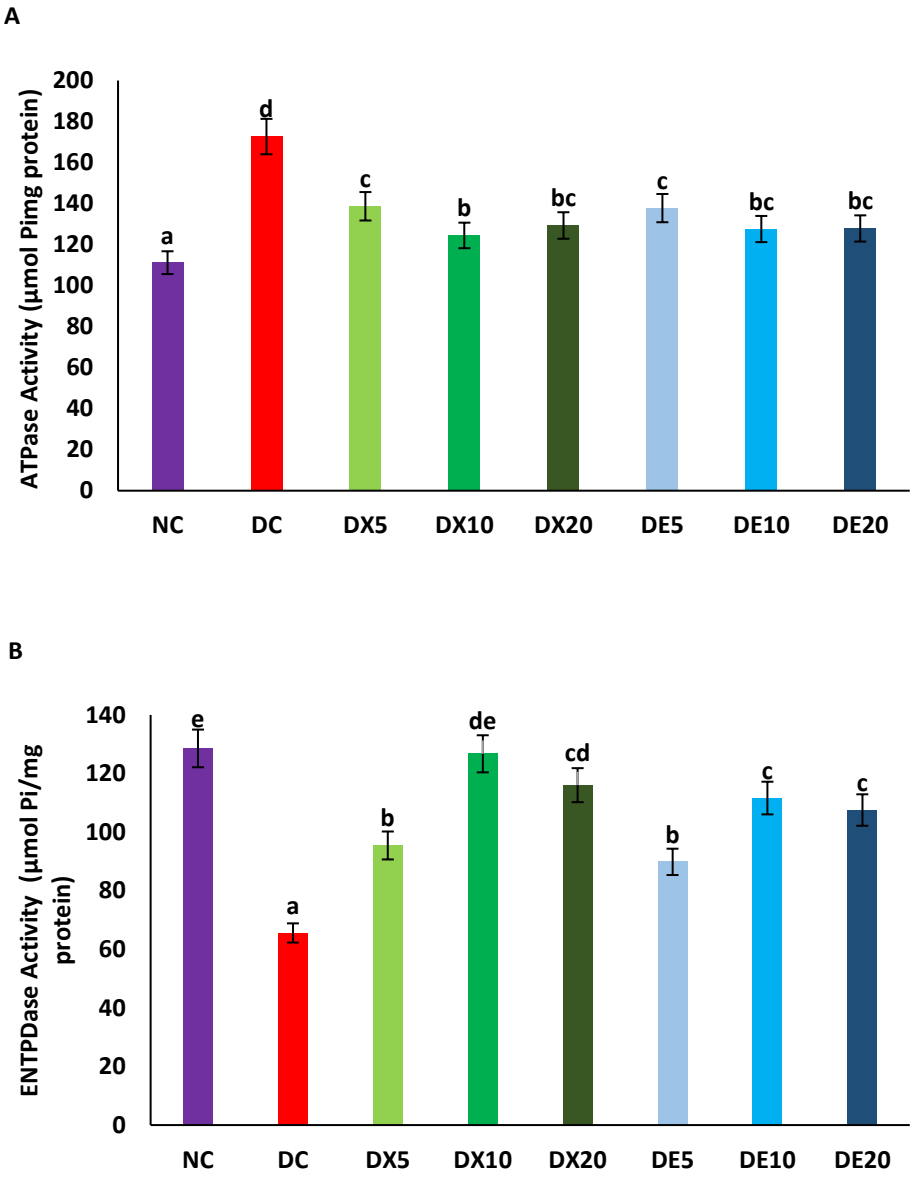

Figure 5.5: **Effect of Xylitol and Erythritol on (A) ATPase activity and (B) E-NTPDase activity in diabetic liver**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE 20). [a-e]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

The induction of T2D significantly ($p<0.05$) elevated the activity of ATPase in liver tissues, while reduced ENTPDase as shown in the DC group in **Fig. 5.5 A-B**. Treatment with xylitol significantly ($p<0.05$) reversed these activities as portrayed by the reduced ATPase activity, and elevated activity of ENTPDase. Although ATPase activities were similarly reduced in all xylitol and erythritol fed groups, the better ENTPDase reducing activity was observed in the DX10 group compared to other treatment groups.

Fig. 5.6 represents the acetylcholinesterase activity of the experimental groups. The DC group significantly ($p<0.05$) elevated acetylcholinesterase activity after the induction of T2D. The treatment groups significantly ($p<0.05$) reduced the activity, when the reductions were significantly lower in the DX10, DX20 and DE10 groups compared to all other groups, which are also comparable with NC group.

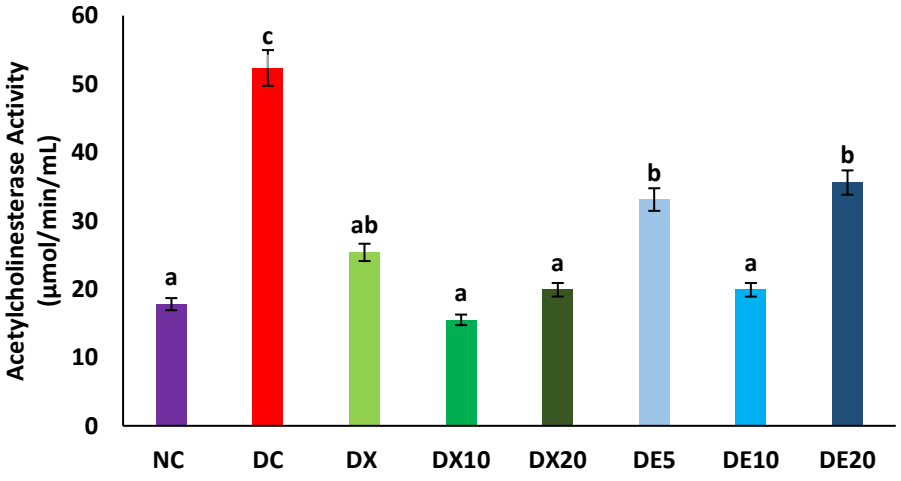

Figure 5.6: **Effects of Xylitol and Erythritol on acetylcholinesterase activity in the liver of diabetic rats at the end of the intervention period**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE 20). [a-c]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

Fig. 5.7 displays the lipase activity of the experimental groups at the end of the intervention period. A significant ($p<0.05$) increase in lipase activity was observed in the DC group when compared to the NC group. The lipase activity was significantly ($p<0.05$) reduced in the hepatic tissues of animals after the treatment with xylitol and erythritol.

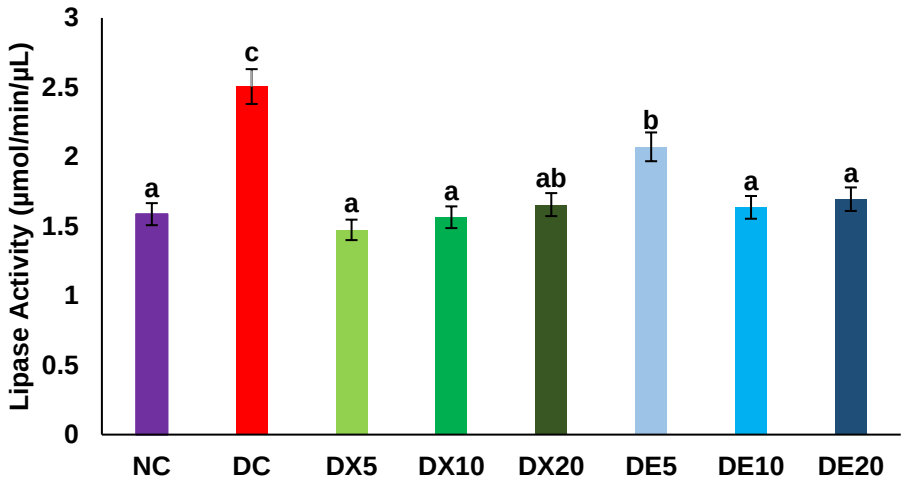

Figure 5.7: **Effect of Xylitol and Erythritol on lipase activity in the liver of diabetic rats**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE 20). [a-c]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

The liver lipid profile data for the experimental groups is presented in **Fig. 5.8**. A significant ($p<0.05$) elevation in total and LDL-cholesterol was observed in the DC group when

compared to the NC group. A significant reduction in total and LDL-cholesterol was shown in the DX10 and DE5 groups respectively, compared to the other treatment groups. The HDL-cholesterol was significantly ($p<0.05$) lower in the DC group which was increased at a great extent in the DX10 group compared to other treatment groups. Induction of T2D also significantly ($p<0.05$) elevated triglyceride level which was significantly ($p<0.05$) lower in the DX20 group compared to the other treatment groups.

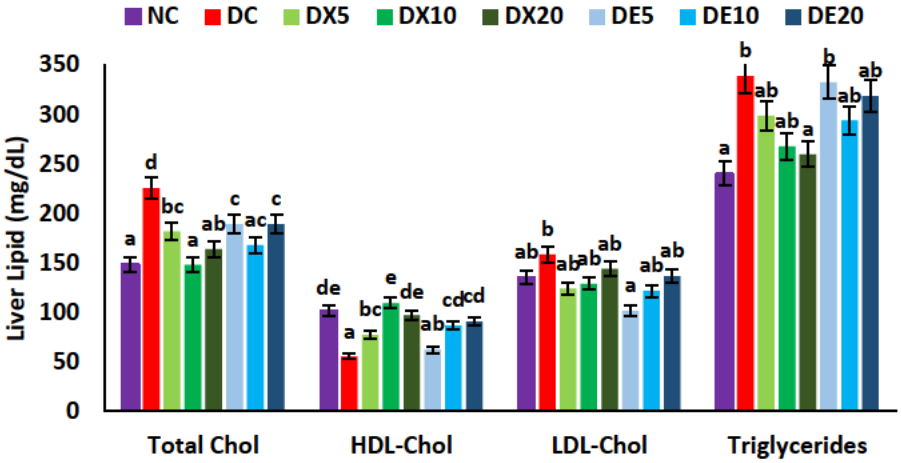

Figure 5.8: **Liver lipid profile of experimental groups**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE 20). [a-d]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

The morphological changes of the hepatic tissues of the experimental groups are depicted in **Fig 5.9**. The NC group revealed normal histo-morphological structures, while the DC group revealed hepatic lesions represented by loss of cellular mass, degeneration of the hepatocytes, dilation of hepatic sinusoids, major cytoplasmic vacuolations and cell death. The hepatic sections from the diabetic animals treated with DX5, DX10 and DE10 showed improved morphology, while the DE5, DX20 and DE20 groups showed slight dilated sinusoids.

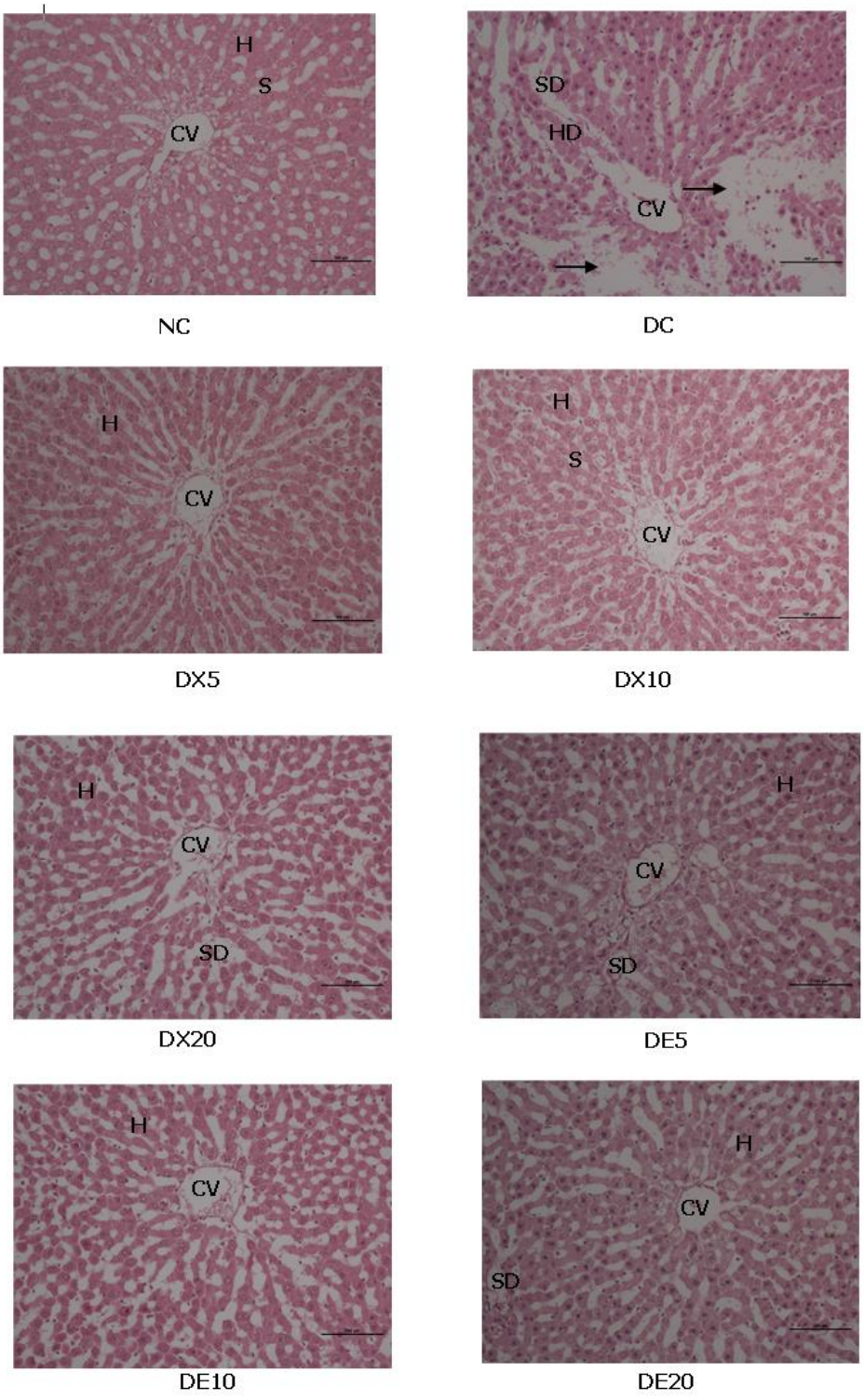

Figure 5.9: **Histological changes in liver in different experimental groups at the end of the intervention period**. Magnification: X400. CV – central vein; H–Hepatocyte; S – Sinusoids; HD – hepatocyte degeneration; SD – hepatic sinusoid dilation; Black arrows – degenerated cells characterized by cytoplasmic vacuolations, loss of cell mass and apoptosis. NC = normal control, DC = diabetic control, DX5 = diabetic xylitol 5%, DX10 = diabetic xylitol 10% , DX20 = diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

5.5 Discussion

Xylitol and erythritol are frequently used sugar alcohols in the food industry as they are perceived to have lower caloric values, glycemic index and insulinemic response (Livesey, 1992). Hence, numerous studies have been undertaken to examine the ability of xylitol and erythritol to regulate blood glucose and insulin for inclusion in diabetic foods and food products (Islam, 2011; Rahman and Islam, 2014; Wölnerhanssen *et al.*, 2019). However, there is still a dearth on their effect on hepatic metabolism in T2D. Therefore, the present study investigated the comparative effects of xylitol and erythritol on T2D induced hepatic injury.

The hepatic tissue conserves normal blood glucose concentrations by storing glucose as glycogen and by generating glucose from glycogen breakdown (Sharabi *et al.*, 2015), when it depends on insulin to manage excess glucose level or hyperglycaemia (Santoleri and Titchenell, 2019). In insulin-resistance state, glycogen deposition from glucose is altered which has been observed in diabetic animals since STZ causes impairment to pancreatic β-cells resulting in insufficient insulin level (Ngubane *et al.*, 2011). In our study, the lower hepatic glycogen level observed in the diabetic control group **(Table 5.1)** indicates that insulin resistance may have altered glycogen synthesis. On the other hand, treatment with xylitol and erythritol was able to restore the liver glycogen when better result was observed in the DX10 group. This indicates the ability of xylitol and erythritol in restoring glycogenesis, thus suggesting their antidiabetic potential.

Oxidative stress plays a pivotal role in the pathogenesis and progression of T2D and its complications (Ighodaro, 2018). This is attributed by hyperglycemia with concomitant redox imbalance which results in dysregulated cellular metabolism (Oguntibeju, 2019). The induction of hepatic oxidative stress as well as the reduction of antioxidative enzymes

activities have been previously reported as a cause of hyperglycemia (Cichoż-Lach and Michalak, 2014; Mohamed *et al.*, 2016). In our study, the induction of T2D led to the lower levels of GSH, SOD and catalase activities, with increased level of MDA in the hepatic tissues **(Figures 5.1-5.4)**. On the other hand, treatment with xylitol and erythritol, significantly ($p<0.05$) elevated GSH level, SOD and catalase activities, with concurrent suppression of MDA level. Our results are in line with results of previously published studies (Kang *et al.*, 2007; den Hartog *et al.*, 2010; Chukwuma and Islam, 2017). This observation indicates the better antioxidative effect of xylitol against hepatic redox imbalance in T2D, compared to erythritol.

Purinergic activities are involved in both the physiology and pathophysiology of the liver. In the presence of insulin resistance these activities cause alteration in the release of nucleotides or uptake of nucleosides, with a decrease in enzyme function of ectonucleotidases (Burnstock *et al*., 2014). The induction of T2D, increased the activity of ATPase and supressed ENTPDase activity in the hepatic tissue of the diabetic animal group **(Fig. 5.5)**, this indicates purinergic dysfunction. To the best of our knowledge the effects of xylitol and erythritol on hepatic purinergic activity has not been conducted in previous studies. Treatment with xylitol and erythritol significantly reversed these activities, with the 10% xylitol group showing a relatively higher effect. These results suggest the potential ability of xylitol and erythritol to improve hepatic purinergic signalling.

Acetylcholinesterase is an enzyme responsible for the inactivation of cholinergic neurotransmission, its elevated activity in the liver has been associated with hepatic dysfunction (Garcia-Ayllon *et al.*, 2012). In the present study, acetylcholinesterase activity increased in hepatic tissues of diabetic control group **Fig. 5.6**, this observation suggests the cholinergic dysfunction due to the increased level of acetylcholine esterase enzyme activity. Some previous studies have also reported the impairment of cholinergic activities triggered

by diabetes (Wahba and Soliman, 1988; Antony *et al.,* 2010). In our study, xylitol and erythritol treated groups significantly ($p<0.05$) reduced the activity of acetylcholinesterase, thus suggesting the improved neurotransmission in the liver.

Lipids are among the essential components in physiological system that manage cellular functions and homeostasis (Ahmed et al., 2020). The liver plays an important role in lipid metabolism including but not limited to various stages of synthesis and transportation of lipids. Therefore, an abnormal lipid profile is to be expected in patients with severe hepatic impairment (Ghadir *et al.,* 2010). In the present study, elevated lipase activity **(Fig. 5.7)** and dysregulated hepatic lipid profile **(Fig. 5.8)** were observed in the diabetic control group. It has been reported that upregulation of hepatic lipase activity is associated with hypertriglyceridemia, reduced HDL levels and high number of small dense LDL (Shina and Kim, 2011), which was observed in the diabetic control group after the induction of T2D in our study. The over accumulation of triglycerides in the hepatocytes causes increased levels of free fatty acids (FFA) which are significant mediators of lipotoxicity (Alves-Bezerra and Cohen, 2017). The reduced lipase activity and restored lipid profile following treatment with xylitol and erythritol indicates their ability to mitigate hepatic lipotoxicity.

In the present study, the histopathology of the liver of the NC group was normal **(Fig. 9)**, with central vein surrounded by vascular channels called sinusoids which transports blood to the central vein from surrounding portal veins and hepatic arteries (Brunt *et al.,* 2014). It also showed intact hepatocyte with well-rounded nucleus and cytoplasm. This finding is in agreement with the description of (Hamdin *et al.,* 2019). Histological examination of the hepatocyte from the diabetic control group showed microscopic changes and morphological disturbances due to streptozotocin administration . The liver section revealed loss of cellular mass, dilated sinusoids, deteriorated hepatocytes, and cytoplasmic vacuolations. These findings of the present study are in agreement with the findings of Gopal et al. (2014) and

Lucchesi et al. (2015) who showed destruction of hepatocytes, dilated sinusoids and a progressive loss of organ structure (Gopal *et al.*, 2014; Lucchesi *et al*., 2015). The improved liver histological organization in the animals treated with xylitol and erythritol indicates their potential therapeutic effect against hepatic dysfunction.

5.6 Conclusion

The data portrays the therapeutic effect of xylitol and erythritol against hepatic dysfunctions and dysmetabolism in T2D rats as portrayed by their ability to extenuate redox imbalance, purinergic and cholinergic dysfunctions, while regulating glycogen content, lipid metabolism and restoring liver morphology. However, xylitol relatively outperformed the activities compared to erythritol in most cases when 10% xylitol group showed better results compared to all other treatment groups in general. Further clinical investigations are required to affirm the findings of this *in vivo* study.

CHAPTER 6

Comparative effects of xylitol and erythritol on redox imbalance, cholinergic dysfunction, purinergic activity, and glucose-lipid metabolic homeostasis in psoas muscles of diabetic rats

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Preface: This article investigated the comparative therapeutic effect of xylitol and erythritol on redox imbalance, purinergic and cholinergic dysfunction, and lipid dysmetabolism in psoas muscle tissue of fructose-streptozotocin (STZ) induced T2D rats. This article is currently under preparation for submission to an international peer-reviewed journal.

6.1 Abstract

The skeletal muscle is the largest organ in the body, it is involved in regulating glucose homeostasis under insulin-stimulated conditions and it is a major site of insulin resistance in type 2 diabetes (T2D). Hence, the present study investigated the therapeutic effect of xylitol and erythritol on redox imbalance, purinergic and cholinergic dysfunction, and glucose-lipid dysmetabolism in muscle tissue of diabetic rats. Seven weeks old male Sprague-Dawley rats were divided into eight groups as follows: Normal control (NC), Diabetic control (DC), Diabetic xylitol 5% (DX5), Diabetic xylitol 10% (DX10), Diabetic xylitol 20% (DX20), Diabetic erythritol 5% (DE5), Diabetic erythritol 10% (DE10) and Diabetic erythritol 20% (DE20). T2D was induced in the diabetic groups and after confirmation of diabetes, the xylitol and erythritol groups were supplied with their respective solutions. The animals were humanely sacrificed after 8 weeks of treatment and their muscle tissues were harvested. Treatment with xylitol and erythritol was able to suppress GSH level and activities of SOD, catalase, and ENTPDase, with concomitant elevation of MDA level and activities of acetylcholinesterase, ATPase, lipase, G6Pase, FBPase, glycogen phosphorylase and amylase. The 10% xylitol treated animals showed significantly ($p<0.05$) better activity in most of the above-mentioned parameters. The muscle histology was further restored after the treatment with xylitol and erythritol. The data of this study suggests the xylitol and erythritol against muscle dysfunction and dysmetabolism, as indicated by its ability to attenuate redox imbalance, purinergic and cholinergic dysfunction, while modulating glucose-lipid metabolism and muscle histology, when the effects of xylitol were better than erythritol and 10% xylitol showed the most beneficial outcomes. Hence, xylitol can be used as a superior supplement over erythritol in antidiabetic foods and food products for the management of T2D.

Keywords: Xylitol, Erythritol, Type 2 diabetes, Muscle dysmetabolism, Glucose uptake

6.2 Introduction

The incidence of type 2 diabetes (T2D) is rapidly increasing and is reaching endemic proportions (Saeedi *et al.*, 2019; Berbudi *et al.*, 2020). It is estimated that 463 million people are currently living with diabetes, with a predicted upsurge to 700 million by 2045 (IDF, 2019b). There are two key features in the pathogenesis of T2D which includes defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond to insulin (Galicia-Garcia *et al.*, 2020). One of the major defects associated with T2D is to utilize glucose properly by peripheral tissues such as the skeletal muscle which is a primary target of insulin-stimulated glucose uptake (Teng and Huang, 2019).

In the postprandial state, 70-90% of glucose uptake occurs in the skeletal muscle which is used for muscle energy production (Jensen *et al.*, 2011; Evans *et al.*, 2019). This alternatively reduces an increase in blood glucose level (Evans *et al.*, 2019). In a diabetic state, glucose uptake by the skeletal muscle is reduced and the muscle uses free fatty acid (FFA) for energy production (Phielix and Mensink, 2008). There is ample evidence showing that prolonged exposure of skeletal muscle to high levels of FFA leads to severe insulin resistance, resulting in oxidative stress (Phielix and Mensink, 2008; Martins *et al.*, 2012). These factors cause the skeletal muscle to undergo structural, functional and metabolic changes, such as impaired glucose uptake, glycogen synthesis, muscle cholinergic dysfunction and impaired muscle glucose-lipid metabolic homeostasis (Martins *et al.*, 2012; Teng and Huang, 2019; Galicia-Garcia *et al.*, 2020).

Studies conducted in skeletal muscle cells, rodents, nonhuman primates, and human subjects have demonstrated that restriction of caloric or protein intake positively mediates insulin sensitivity (Zhang *et al.*, 2021). The use of sugar alcohols as diabetic supplements have shown to improve insulin sensitivity in a number of previous studies as well (Livesey, 2003;

Wölnerhanssen *et al.*, 2019). Sugar alcohols are nutritive sugar substitutes that have lower caloric content and glycemic index compared to sugar (sucrose), hence their use as sugar alternatives has been rapidly increased in the recent years (Malgorzata Grembecka, 2018). Among many sugar alcohols, the usefulness of xylitol and erythritol as sugar alternatives on diabetes has been extensively studied (Wölnerhanssen *et al.*, 2019). A previous study reported that xylitol exerts glucose lowering effects via reducing carbohydrate digestion and intestinal glucose absorption, with an apparent increase in muscle glucose uptake (Chukwuma and Islam, 2015). A number of studies conducted on animals imply that xylitol has potential insulin-sensitizing effects (Salminen *et al.*, 1989; Islam and Indrajit, 2012). It has been also shown to improve glycaemic control (Islam, 2011) and pancreatic islets morphology (Rahman and Islam, 2014), and has the ability to ameliorate diabetes related oxidative stress (Chukwuma and Islam, 2017b). Erythritol on the other hand has been reported to exhibit an endothelium protective effect (den Hartog *et al.*, 2010), postprandial glucose-lowering effects and improving glycaemic control in diabetic rats (Chukwuma, Mopuri, *et al.*, 2018). A previous study reported that a single dose of 75 g of erythritol in 300 ml of water modulates gut hormone (GPL-1) and delays gastric emptying in lean and obese subjects (Wölnerhanssen *et al.*, 2016).

Although numerous studies have been conducted on xylitol and erythritol in terms of their hypoglycemic and antidiabetic properties, particularly in terms of skeletal muscle glucose metabolism, it is still not clear which one is better in this regard. Thus, the present study was conducted to examine the comparative therapeutic effect of xylitol and erythritol on redox imbalance, purinergic and cholinergic dysfunction, and glucose-lipid dysmetabolism in psoas muscle tissue of fructose-streptozotocin (STZ) induced T2D rats.

6.3 Materials and methods

Kindly refer to Chapter 2; 2.6 (sub sections 2.6.1-2.6.3, 2.6.5, 2.6.8-2.6.13) and 2.8 for further details.

6.4 Results

Fig. 6.1 displays the effect of xylitol and erythritol on glutathione (GSH) concentration in diabetic muscle tissue. Significantly ($p<0.05$) reduced level of glutathione was observed in the diabetic control group compared to the normal group. Treatment with xylitol and erythritol was able to reverse this depletion of glutathione, when 10% xylitol fed group showing the most significant ($p<0.05$) increase compared to other treatment groups. The level of glutathione in DX20, DE10 and DE20 groups were significantly higher than DC, DX5 and DE5 groups as well.

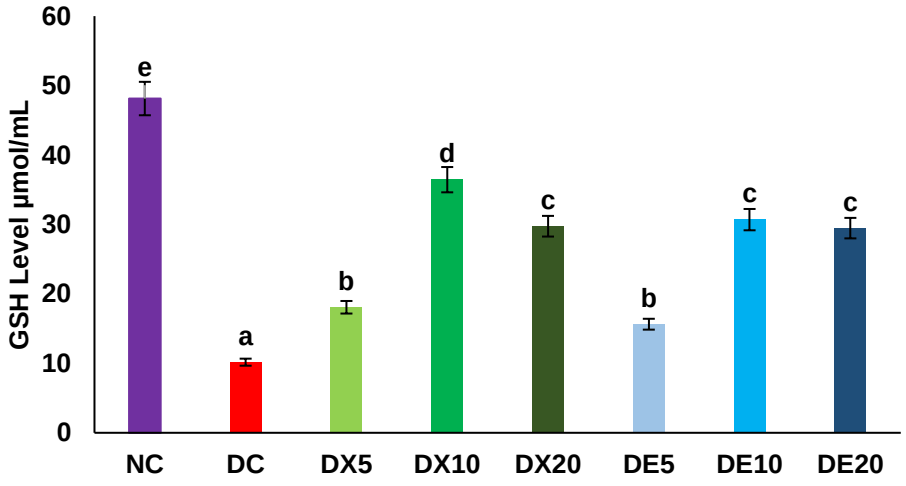

Figure 6.1: **Effect of Xylitol and Erythritol on GSH level in the psoas muscle at the end of the intervention trial**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). [a-e]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

Fig. 6.2 displays the effect of xylitol and erythritol on malondialdehyde (MDA) level in diabetic muscle tissue. The induction of T2D significantly elevated the level of MDA as observed in the DC group when compared to the NC group. Treatment with xylitol and erythritol was able to significantly ($p<0.05$) supress the level of MDA, with the DX 10% group showing the greatest reduction which was the same as that of the NC group. Other treatment groups also have significantly lower levels of MDA, when the results of DX20 and DE10 groups were significantly lower than the DX5, DE5 and DE20 groups.

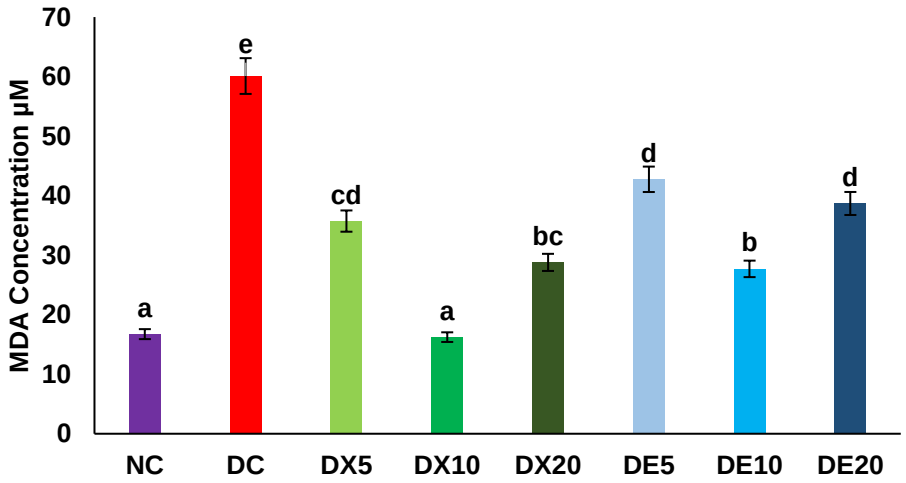

Figure 6.2: **Effect of Xylitol and Erythritol on MDA level in the psoas muscle at the end of the intervention trial.** Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). [a-e]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

Fig. 6.3 displays the effect of xylitol and erythritol on superoxide dismutase (SOD) activity in diabetic psoas muscle tissue. The SOD activity was significantly ($p<0.05$) reduced in the diabetic control group compared to the normal control group. The enzyme activity

significantly ($p<0.05$) increased upon treatment with both xylitol and erythritol, with a significant increase observed in the DX 10% group compared to the other treatment groups.

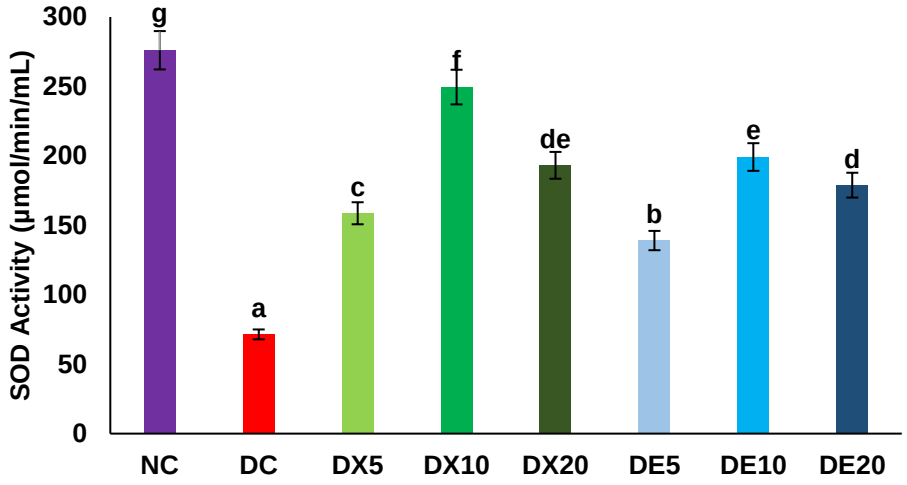

Figure 6.3: **Effect of Xylitol and Erythritol on SOD activity in the psoas muscle at the end of the intervention trial**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). $^{a-g}$Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

Fig. 6.4 presents the data of the effect of xylitol and erythritol on catalase activity in the diabetic psoas muscle tissue. Depletion of catalase activity was observed after the induction of T2D as shown by the DC group when compared to the NC group. The treatment groups were able to significantly ($p<0.05$) increase the enzyme activity. The diabetic xylitol 10% group showed a significant ($p<0.05$) increase in the enzyme activity compared to all other treatment groups.

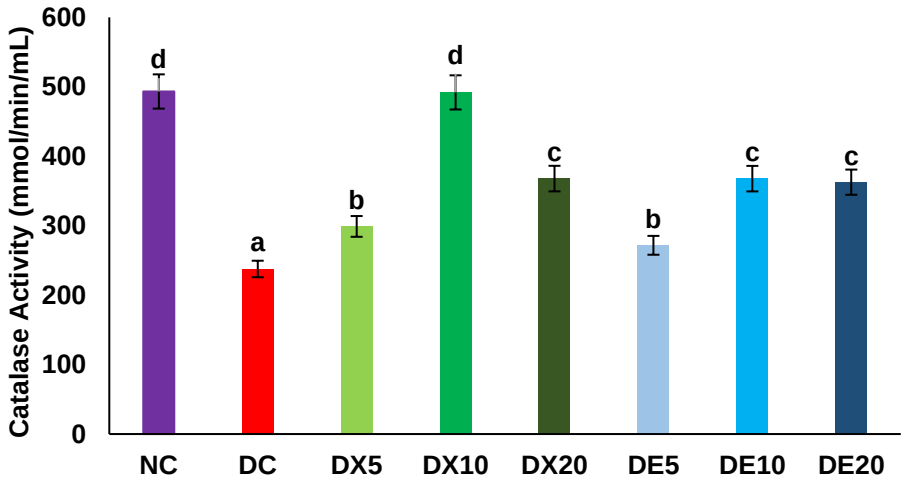

Figure 6.4: **Effect of Xylitol and Erythritol on catalase activity in the psoas muscle at the end of the intervention trial.** Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). [a-e]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

The induction of T2D significantly ($p<0.05$) elevated the activity of ATPase and reduced the activity of ENTPDase in the psoas muscle tissue, as observed in the diabetic control group compared to normal control group, **Fig. 6.5**. While DX10, DX20 and DE10 groups showed significantly ($p<0.05$) lower ATPase activity compared to DC group, the highest reduction was observed in the DX10 group, which was comparable with NC group. No significant difference was observed between the DX5, DE5 and DE20 groups compared to the DC group.

The activity of muscle ENTPDase was significantly ($p<0.05$) decreased after the induction of T2D as shown in DC group compared to the NC group (**Fig. 6.5B**). Treatment with xylitol and erythritol significantly improved the level of ENTPDase when better results were observed in the DX10 and DE10 groups compared all other treatment groups. The results of these two groups were also comparable with NC group.

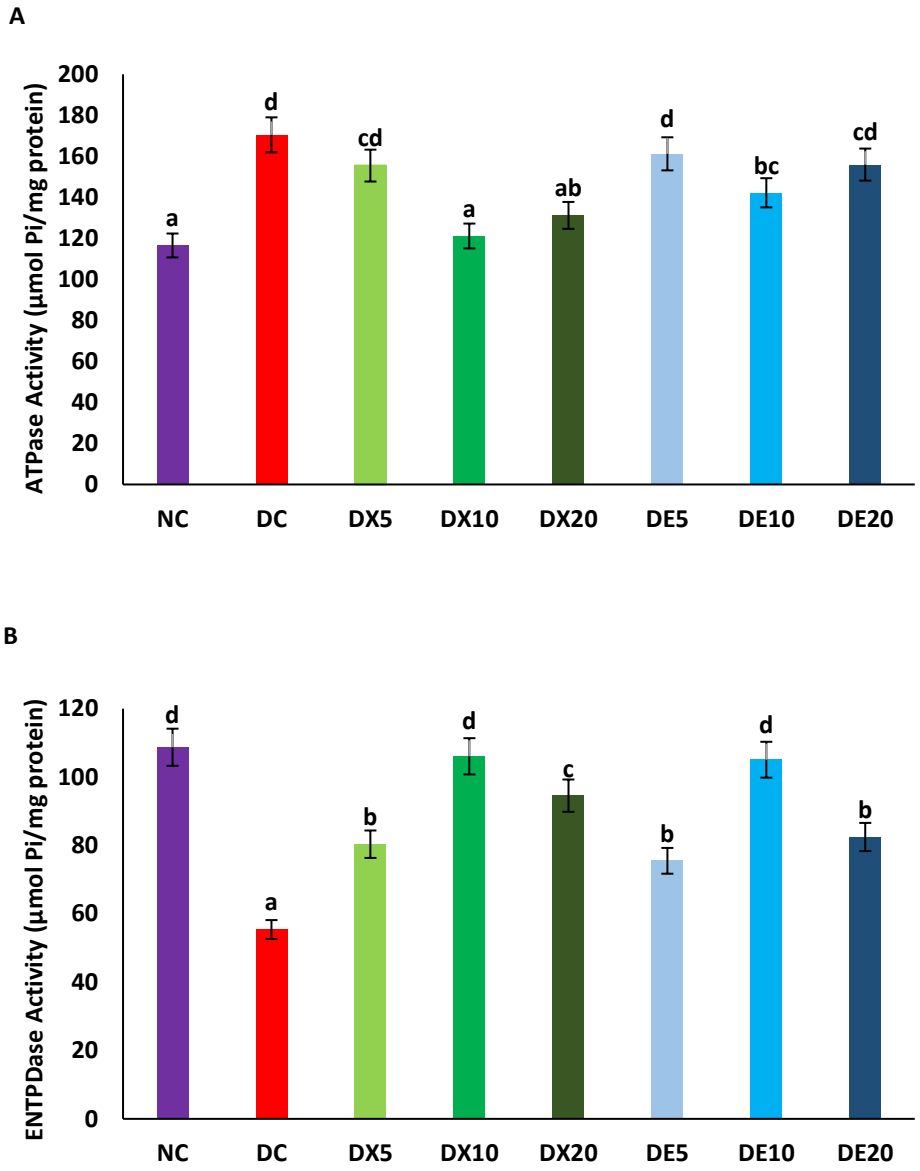

Figure 6.5: **Effect of Xylitol and Erythritol on (A) ATPase activity and (B) E-NTPase activity in diabetic psoas muscle**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). [a-d]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

Fig. 6.6 displays the effect of xylitol and erythritol on muscle acetylcholinesterase activity of the experimental animals. The activity of acetylcholinesterase was significantly ($p<0.05$) increased in the DC group compared to the NC group. The treatment with xylitol and

erythritol significantly (<0.05) reduced the activity, when better results were observed for DX10, DX20 and DE10 groups compared to all other treatment groups. There was no significant difference between the results of DX10 and NC groups.

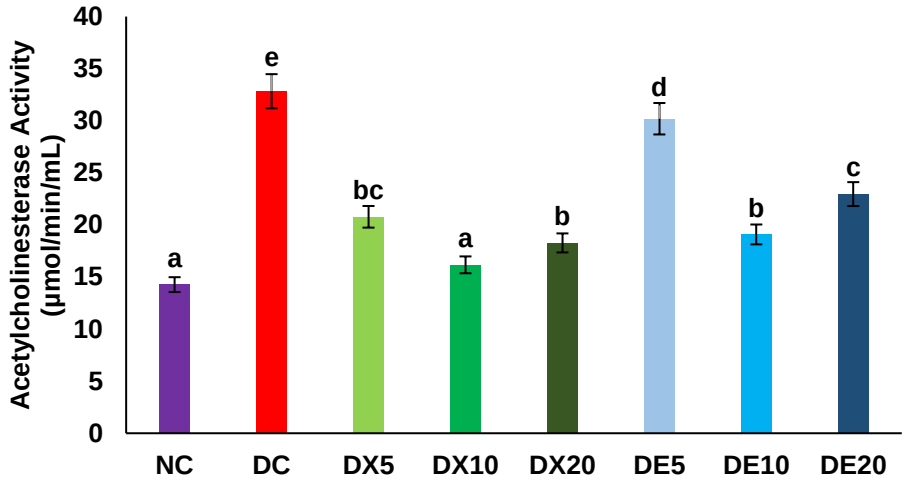

Figure 6.6: **Effect of Xylitol and Erythritol on acetylcholinesterase activity in diabetic psoas muscle**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). [a-e]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

A significant ($p<0.05$) increase in lipase activity in the muscle was observed in the DC group compared to the NC group, **Fig. 6.7**. The lipase activity was significantly ($p<0.05$) reduced in the muscle tissues of the treatment groups after treating with xylitol and erythritol. While the lipase activity in DX10, DX20, DE10 and DE20 groups were significantly lower than the DC group, DX10 group showed better results compared to all other treatment groups. There was no significant difference among the lipase activity of DX5, DE5 and DC groups.

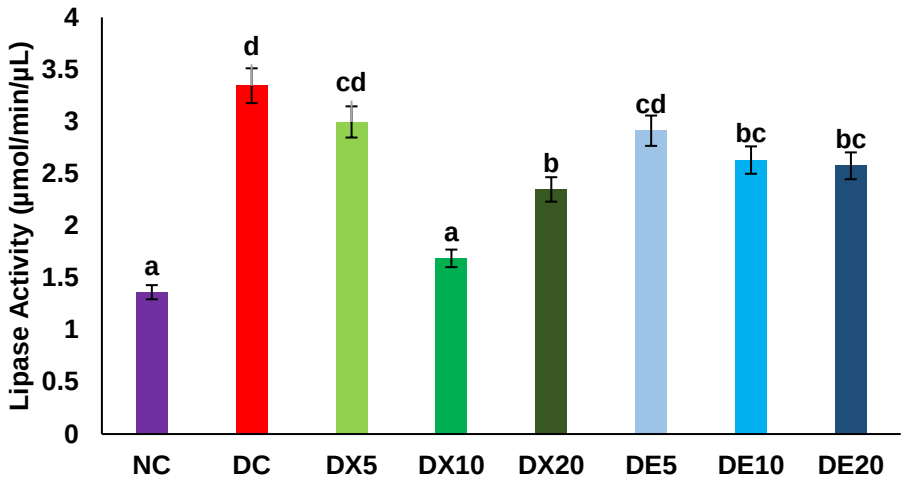

Figure 6.7: **Effect of Xylitol and Erythritol on lipase activity in diabetic proas muscle**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). [a-d]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

The effects of xylitol and erythritol on carbohydrate metabolizing enzymes activities are presented in **Fig.s 6.8 – 6.11**. The muscle glucose-6-phosphatase (G6Pase), fructose-1,6-bisphosphatase (FBPase), glycogen phosphorylase and amylase activities were significantly ($p<0.05$) elevated after the induction of T2D as shown in the DC group. These activities were inhibited significantly ($p<0.05$) after the treatment with xylitol and erythritol. Although not much difference was observed for glucose-6-phosphatease activity among the treatment groups, the activity of fructose-1,6-bisphosphatase, glycogen phosphorylase and amylase activities were significantly reduced in the DX10 compared to all other treatment groups. The results of this group are also comparable with NC group.

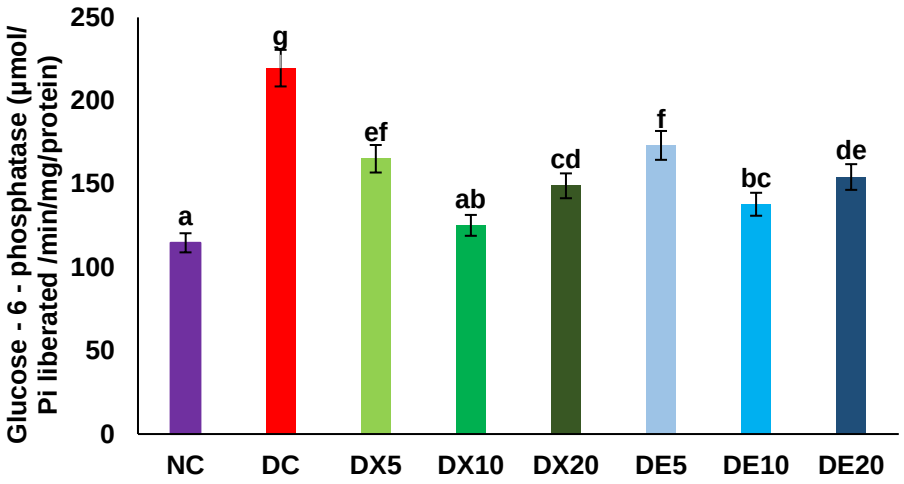

Figure 6.8: **Glucose – 6 – phosphatase activity of experimental animals**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). [a-g]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

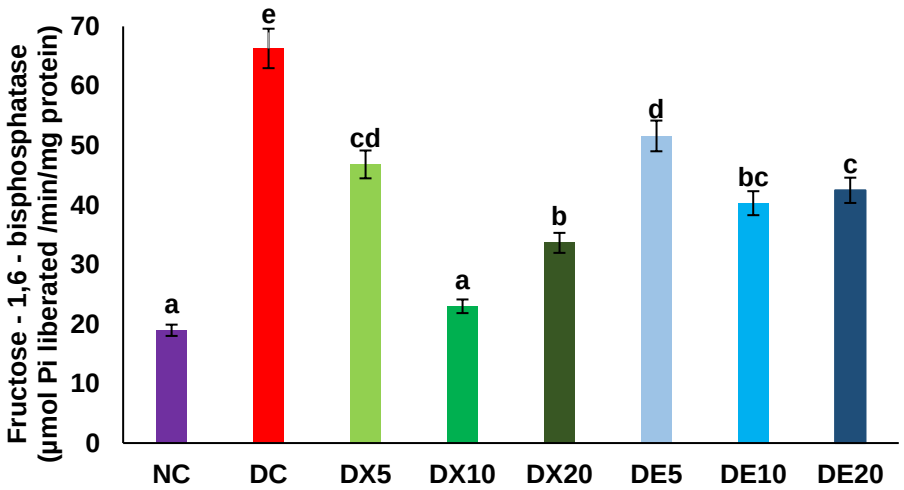

Figure 6.9: **Fructose – 1,6 – bisphosphatase activity of experimental animals**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). [a-e]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

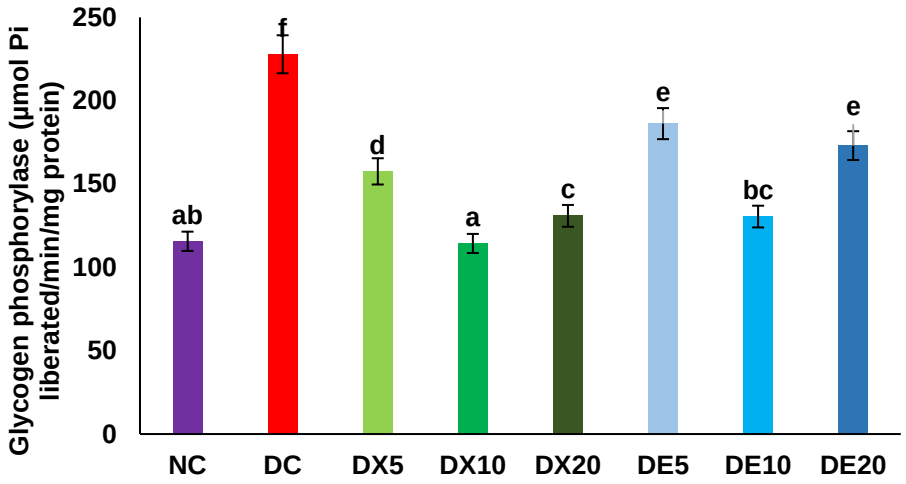

Figure 6.10: **Glycogen phosphorylase activity of experimental animals**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). [a-f]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

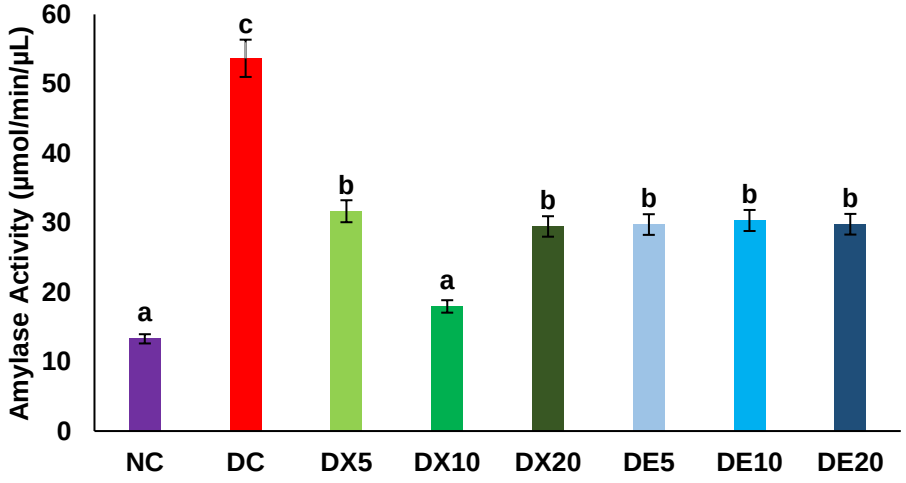

Figure 6.11: **Glycogen phosphorylase activity of experimental animals**. Values = mean ± SD; n = 7 (NC), n = 6 (DX10 and DX20), 5 (DC, DX5, DE5 and DE10) and n=2 (DE20). [a-c]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%.

In **Fig. 6.12**, the normal group reveals the transverse section of healthy muscle histology with homogenously dispersed muscle fibres containing myocyte nuclei located at the periphery which are intactly surrounded by endomysium. The diabetic group shows deteriorated muscle histology with degenerated muscle fascicles and enlarged endomysium infiltrated by myocyte nuclei. Treatment with both xylitol and erythritol at 5 %, 10 % and 20 % concentrations restored muscle histology with mostly improved muscle fascicles which are delineated by endomysium, when better results were obtained for 10% xylitol fed groups compared to all other treatment groups.

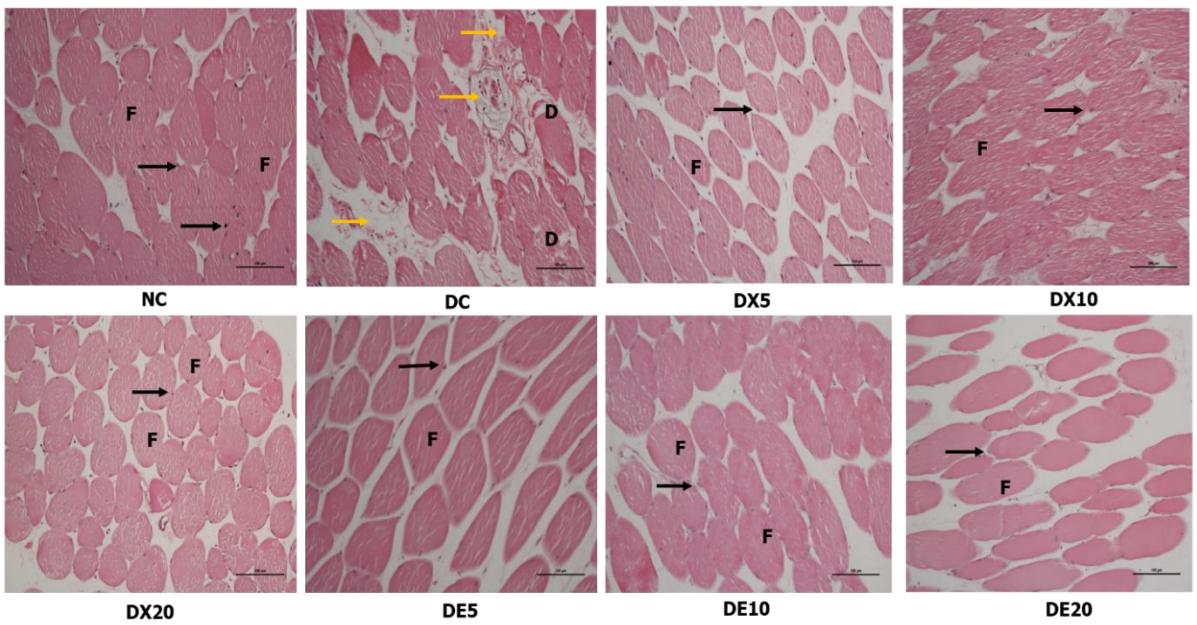

Figure 6.12: Histological alterations of muscle of experimental groups. Magnification: X200. NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 =Diabetic erythritol, DE10 = Diabetic erythritol 10% and DE20 =Diabetic erythritol 20%. F- healthy muscle fascicles, D- degenerated muscle fascicles, Black arrow – nuclei, Yellow arrow – enlarged endomysium.

6.5 Discussion

The skeletal muscle is one of the most important organs affected by insulin resistance, as it plays an essential role in glucose homeostasis (Phielix and Mensink, 2008). Therefore,

currently available glucose-lowering therapies are targeted at improving muscle function, muscle glucose uptake and utilization (Chaudhury *et al.*, 2017; Naimi *et al.*, 2017). Although numerous studies have reported the antidiabetic properties of xylitol and erythritol, there is still a dearth on their effect on muscle metabolism in T2D. Therefore, this study comparatively investigated the effect of xylitol and erythritol in modulating redox imbalance, purinergic and cholinergic dysfunction, and improving glucose-lipid dysmetabolism in psoas muscle tissue of fructose-fed streptozotocin (STZ)-induced type 2 diabetic rats.

Hyperglycemia induced oxidative stress is one of the key pathomechanism linked to the development and progression of T2D (Oguntibeju, 2019). The incidence of oxidative stress has been reported as a cause of mitochondrial dysfunction in skeletal muscle in diabetic state (Yokota *et al.*, 2009). In the present study oxidative stress is shown by the reduced levels of GSH, SOD and catalase activities, as well as the increased level of MDA in the muscle tissues of the DC group. These changes have been previously reported in skeletal muscle damage caused by hyperglycemic-induced oxidative stress (Samir *et al.*, 2018). Treatment with xylitol and erythritol elevated GSH level, SOD and catalase activities, and supressed the level of MDA, this indicates antioxidative effect of the sugar alcohols. Similar results have been reported from previous studies (Kang *et al.*, 2007; den Hartog *et al.*, 2010; Chukwuma and Islam, 2017). However, animals treated with 10% xylitol significantly reversed the antioxidant status the most compared to the other treatment groups. This observation indicates that xylitol is a better antioxidant compared to erythritol.

Purinergic receptors are present in most tissues and are involved in various signalling pathways, including the microcirculation in skeletal muscles (Bornø *et al.*, 2012). These receptors are activated by adenine nucleic acids which are activated specifically by adenine (Burnstock and Novak, 2013). Purinergic enzymes catalyses these reactions and their altered activities have been implicated in muscle dysfunction leading to impaired contraction and

glucose uptake (Kim *et al.*, 2002; Juel *et al*., 2014). The induction of T2D lead to increased activity of ATPase and supressed ENTPDase activity in muscles of the diabetic animals (Fig. 6.5), indicating the incidence of purinergic dysfunction which implies a decline in the levels of ATP and adenosine in the muscles. These alterations also imply modifications also imply a disruption in the energy metabolism of the muscle (Bianco and McAninch, 2013; Oizel *et al.*, 2020). To the best of our knowledge, it is the first time we examined the effects of xylitol and erythritol on muscle purinergic activity and no previous studies were conducted on these parameters before. In the present study, therapeutic effect of xylitol and erythritol on purinergic dysfunction in diabetic muscle is shown by the decreased ATPase activity and elevated ENTPDase activity. The reversed activities suggest improved energy homeostasis and muscle glucose uptake.

The function of acetylcholinesterase is to hydrolyze acetylcholine and terminate impulse transmissions at cholinergic synapses (Rosenberry, 1975). It is expressed during apoptosis induced by various stimuli including the development of diabetes (Sánchez-Chávez and Salceda, 2000; Zhang *et al.*, 2012). In the present study, the increased acetylcholinesterase activity in the muscle tissues of the diabetic animals (Fig. 6.6), implies a decline in the level of acetylcholine indicating cholinergic dysfunction. The impairment of cholinergic activities generated by diabetes has been previously reported (Wahba and Soliman, 1988; Antony *et al.*, 2010). In our study treatment with xylitol and erythritol significantly ($p<0.05$) reduced the activity of acetylcholinesterase, with 10% xylitol treated group showing the most reduction. This observation suggests increased levels of acetylcholine which implies improved muscular neurotransmission and glucose uptake. Thus, implying enhanced muscle cholinergic function.

Skeletal muscle is the major site for disposal of postprandial glucose in healthy normal glucose tolerance individuals (DeFronzo, 2004; Defronzo, 2009). The ingested glucose

triggers the pancreas to release insulin and the increased plasma insulin level stimulates glucose uptake in the skeletal muscle (Alvim *et al.*, 2015; Honka *et al.*, 2018). In insulin resistance states such as T2D, the disposal of glucose in skeletal muscle is impaired (Wilcox, 2005). This is due to impaired insulin signalling and various post-receptor intracellular defects such as impaired glucose transport and glucose phosphorylation, and reduced glucose oxidation and glycogen synthesis (Bajaj and DeFronzo, 2003; Karlsson and Zierath, 2007). In the present study, the glycolytic flux was impaired in muscles of T2D as shown by elevated fructose-1,6-bisphosphatase (FBPase) activity (**Fig. 6.9**). FBPase is the key enzyme in gluconeogenesis which catalyses the hydrolyses of fructose 1,6-biosphosphate to fructose 6-phosphate (Timson, 2019). The impairment is also indicated by glycogenolytic effect as shown by the elevated activities of G6Pase, glycogen phosphorylase and amylase (**Fig, 7.8, 7.10 and 7.11**), as these enzymes are responsible for the breakdown of glycogen to glucose (van Schaftingen and Gerin, 2002; Jensen *et al.*, 2011; Brust *et al.*, 2020). The elevated gluconeogenic and glycogenolytic fluxes also indicate a decline in ATP generation which implies an impaired energy homeostasis (Berg et al., 2001). This is correlated by the altered purinergic enzyme activities (**Fig. 6.5**). Treatment with xylitol and erythritol was able to decrease the activity of the carbohydrate metabolizing enzymes activities, which corresponds to results reported from previous studies (Wölnerhanssen *et al.*, 2019; Ahuja *et al.*, 2020), however the 10% xylitol group showed the most effect. This observation indicates the potential ability of xylitol and erythritol to improve glycolysis, glycogenesis and energy homeostasis.

In T2D, decreased fat oxidation capacity and high levels of circulation free fatty acids (FFAs) play a role in the development of the condition (Randle *et al.*, 1963). FFAs in the skeletal muscle causes insulin resistance by reducing insulin stimulated glucose uptake, through the accumulation of lipid inside the muscle (Boden, 1999; Phielix and Mensink, 2008). In the

present study, elevated lipase activity (**Fig. 6.7**) was observed in the diabetic control group which is an indication of dysregulated lipid metabolism. Treatment with xylitol and erythritol, in particular the 10% xylitol group significantly ($p<0.05$) reduced lipase activity. Previous animals studies have reported on the ability of xylitol and erythritol in improving lipid metabolism (Yokozawa, Kim and Cho, 2002; Amo *et al.*, 2011; Chukwuma and Islam, 2016; Kawano *et al.*, 2021). Thus, suggesting the potential ability of the sugar alcohols to improve muscle lipid metabolism which implies a metabolic switch to glucose for muscle energy production which correlates with the decreased glycogenolysis, and gluconeogenesis as depicted by the reduced activities of G6Pase, FBPase and glycogen phosphorylase (**Fig. 6.8 – 6.10**).

The histological changes in the muscle of the diabetic control group (**Fig. 12**), is shown by degenerated muscle fascicles and enlarged endomysium infiltrated by myocyte nuclei. These histological modifications may imply the occurrence of myopathy and atrophy which commonly associated with diabetic complication due to hyperglycemia and insulin resistance (Hernández-Ochoa and Vanegas, 2015; Perry *et al.*, 2016). Treatment with xylitol and erythritol was able to restore muscle histology, this indicates beneficial effect of the sugar alcohols against muscle dysfunction in T2D.

6.6 Conclusion

The data from the present study suggest the therapeutic effect of xylitol and erythritol against muscle dysfunctions and dysmetabolism instigated by T2D, as shown by their ability to regulate redox imbalance, purinergic and cholinergic dysfunctions, as well as modulating glucose-lipid metabolism and muscle histology. However, 10% xylitol had a better effect against muscle dysfunction and dysmetabolism compared all other doses of xylitol and erythritol. Thus, suggesting xylitol as a better supplement for the management of T2D over

erythritol when 10% dietary dose can be used for the most effective health benefits. However, further clinical studies are recommended to ascertain these findings.

CHAPTER 7

Comparative effects of xylitol and erythritol on gastric emptying, digesta transit, intestinal glucose absorption and blood glucose in type 2 diabetic rats

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Preface: This article reports the comparative effects of xylitol and erythritol on gastric emptying, digesta transit, intestinal glucose absorption and blood glucose in a type 2 diabetes model of rats. The manuscript of this chapter is currently under preparation for submission to an international peer-reviewed journal.

7.1 Abstract

Previous studies have reported that xylitol and erythritol possess hypoglycemic and antidiabetic potentials, however it is not clear which one is a better in terms of their effects on gastric emptying, digesta transit, intestinal glucose absorption and blood glucose levels. The present study comparatively investigated the effects of xylitol and erythritol on above-mentioned parameters in a type 2 diabetes model of rats. After an overnight fast, the experimental animals were oral dosed with either xylitol (1 g/kg bw) or erythritol (1 g/kg bw), containing glucose and phenol red (0.05%) as a recovery marker, when control animals were ingested with vehicle and phenol red only. Exactly one hour after the ingestion, animals were humanely sacrificed and the whole gastrointestinal tract was harvested and snap frozen in liquid nitrogen for further analyses. Blood glucose of the animals in each group was measured just before oral dosing and sacrificing, respectively. Both sugar alcohols treated animals were able to reduce small intestinal glucose absorption and postprandial blood glucose increase, while delaying gastric emptying and increased digesta transit, when the results were better for xylitol ingested groups compared to erythritol treated animals in terms of all parameters analysed in this study. The data of this study suggest that xylitol and erythritol exerted hypoglycemic effects possibly by delaying gastric emptying time, increasing digesta transit and reducing intestinal glucose absorption, when the results were better for xylitol compared to erythritol ingested group. However, further clinical studies are required to ascertain the results of this pre-clinical study.

Keywords: Xylitol, Erythritol, Type 2 diabetes, Glucose absorption, Gastric emptying

7.2 Introduction

The growing pandemic of diabetes poses a major public health challenge for almost every country across the globe (Lovic *et al.*, 2020). According to the International Diabetes Federation (IDF), about 463 million people have diabetes worldwide and this figure is expected to increase by 51% in 2045 (IDF, 2019b). Among the types of diabetes, type 2 diabetes (T2D) is the most prevalent one which accounts for more than 90% of all diabetic cases (Laakso, 2019). T2D is a heterogeneous disorder characterised by relative insulin deficiency caused by partial pancreatic β-cell dysfunction and insulin resistance in target organs (Galicia-Garcia *et al.*, 2020). It is influenced by several factors such as a sedentary lifestyle, obesity and high calorie diet intake (Adeva-Andany *et al.*, 2019; Laakso, 2019).

The excessive dietary consumption of sucrose- and fructose-containing foods and food products has been associated with numerous health problems such as cardio-metabolic diseases, overweight, insulin resistance, lipid dysregulation, visceral adiposity, and the risk of developing T2D (Ley *et al.*, 2016; Azaïs-Braesco *et al.*, 2017; Vreman *et al.*, 2017). Dietary modification has been greatly encouraged for diabetic individuals, therefore, there has been a growing interest in using nutraceuticals for the management of T2D which includes sugar alcohols (Edwards *et al.*, 2016; Wölnerhanssen and Meyer-Gerspach, 2019).

Sugar alcohols are a class of sugar substitutes that have gained approval for use by obese and diabetic individuals due to their integrated blood glucose lowering effects compared to table sugar (Mäkinen, 2011; Grembecka, 2015). Xylitol and erythritol are among the popular sugar alcohols in the market (Malgorzata Grembecka, 2018). A number of previous studies have reported that xylitol and erythritol have potential beneficial effects in managing diabetes and its related metabolic complications (Wölnerhanssen *et al.*, 2019; Msomi *et al.*, 2021). In a previous study, the supplementation of 10% dietary xylitol for 3 weeks significantly reduced

non-fasting blood glucose and serum fructosamine levels, enhanced the serum insulin level and improved glucose tolerance ability compared to 10% sucrose in non-diabetic rats (Islam, 2011). In another study, it was reported that supplementation of 1 or 2 g of xylitol per 100 kcal diet for 8 weeks significantly reduced visceral fat mass and plasma lipid concentration in high fat diet-fed rats (Amo *et al.*, 2011). Xylitol has also been suggested to exhibit potential hypoglycemic and antidiabetic effects by decreasing carbohydrate digesting enzyme activities and intestinal glucose absorption, as well as delaying gastric emptying, intestinal digesta transit and muscle glucose uptake in normal and type 2 diabetic rats (Chukwuma and Islam, 2015). On the other hand, in a recent study the ingestion of erythritol between 10 and 50 g in lean volunteers stimulated GI hormone release (CCK, aGLP-1 and PYY) and delayed gastric emptying, with no effect on blood glucose, insulin, glucagon, motilin or GIP release, blood lipids or uric acid concentrations (Wölnerhanssen *et al.*, 2021). A study by Flint et al. (2014) reported that oral administration of 30 g/day erythritol for 4 weeks improved arterial endothelial in type 2 diabetic subjects (Flint *et al.*, 2014). In diabetic rats, the oral administration of 100-400 mg/kg bw of erythritol for 10 days significantly decreased blood glucose level and oxidative stress biomarkers such as lipid peroxidation and protein glycosylation in the kidney, liver and serum (Yokozawa, Kim and Cho, 2002).

Despite the above-mentioned potential hypoglycemic and antidiabetic effects of xylitol and erythritol, further investigation is still required to ascertain which sugar alcohol is better for use as a supplement in diabetic foods and food products. Hence, the present study was conducted to examine the comparative effects of xylitol and erythritol on gastric emptying, digesta transit, intestinal glucose absorption and blood glucose level in a fructose-fed streptozotocin (STZ)-induced T2D model of rats.

7.3 Materials and methods

Kindly refer to Chapter 2; section 2.7 and 2.8 for further details.

7.4 Results

Phenol red was used as a recovery marker to estimate the effects of xylitol and erythritol on in vivo gastric emptying, digesta transit and glucose absorption in the different parts of small and large intestine. The mean total amount of phenol red recovered from the entire gastrointestinal tract (GIT) of all the experimental groups was 91.31 ± 2.64%, which was expressed as a percentage of the total amount of phenol red given to the animals.

Fig. 7.1 represents the distribution of phenol red across the different segments of the GIT. Phenol red was higher in the stomach followed by cecum, last two quarters and first two quarters of the small intestine and colon, however it was lower in the proximal end of the small intestine and colon compared to other parts of the GIT. The phenol red recovered in the stomach was significantly ($p<0.05$) higher for the Diabetic Xylitol (DX) and Diabetic Erythritol (DE) groups compared to the other groups. The phenol red distribution of the Diabetic Control (DC) group was significantly ($p<0.05$) higher in the first and second quarters compared to the other groups. In the third and fourth quarters, the phenol red distribution was significantly ($p<0.05$) higher in the Normal Control (NC) group compared to the other groups. In the cecum, the Diabetic Acarbose (DA) group had significantly ($p<0.05$) higher phenol red recovered compared to the other groups. In the proximal colon, phenol red recovered was relatively higher in the NC, DC and DA groups compared to the DX and DE groups, however in the distal colon phenol red distribution was relatively lower in DX group compared to the other groups.

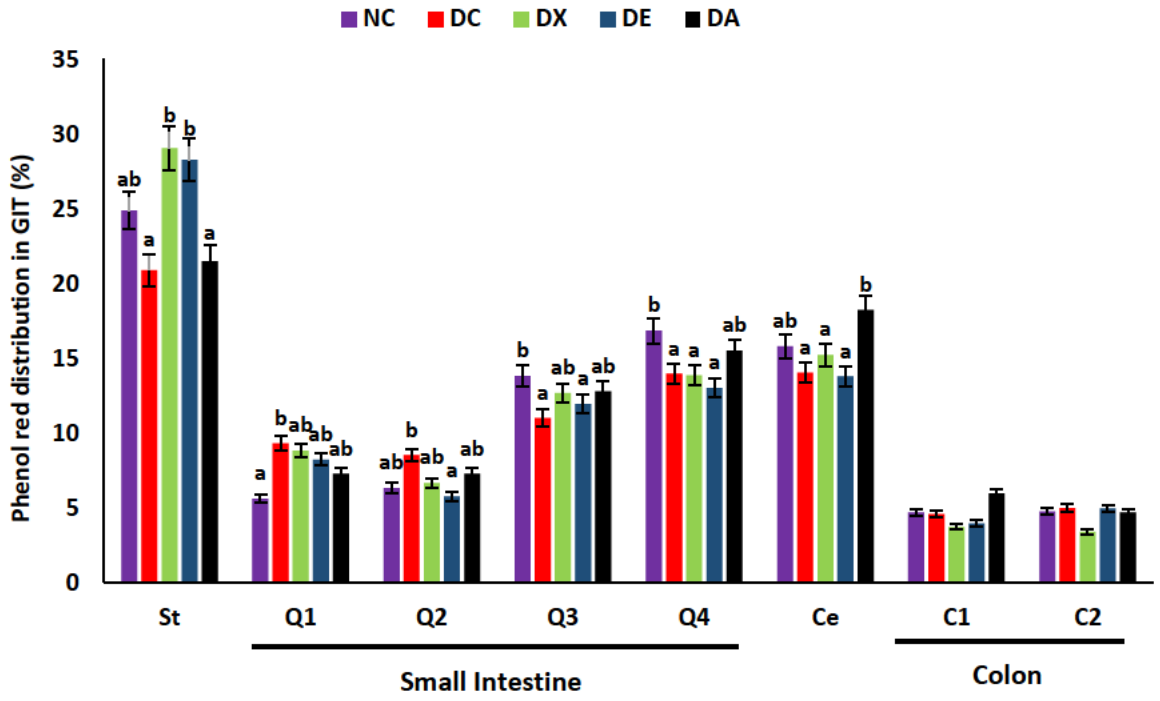

Figure 7.1: Data showing the distribution of phenol red (marker) in the different segments of the GIT at 1 hour after the dose ingestion. Values = mean ± SD; n = 6 (NC, DC, DX, DE and DA).[a-b]Different letters presented above the bars for a given group are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX = Diabetic xylitol, DE =Diabetic erythritol and DA = Diabetic acarbose

The data for the final glucose concentrations and blood glucose increase in animals after 1 h of the dose administration is presented in **Fig. 7.2**. Blood glucose level was significantly ($p<0.05$) lower in the normal control group compared to the other groups. The ingestion of xylitol was able to relatively lower blood glucose increase when compared to the DC, DE and DA groups.

The intestinal glucose absorption index (GAI) ais presented in **Fig. 7.3**. In the first and second quarters of the small intestine, as well as the distal colon, the GAI of the DX and DA groups was significantly ($p<0.05$) lower compared to the NC, DC and DE groups. In the third and fourth quarters the GAI of the DX group was significantly ($p<0.05$) lower compared to

all the other groups. In the cecum segment, the NC and DX groups displayed significantly ($p<0.05$) lower GAI compared to DC, DE and DA. In the proximal colon, the DA group showed significantly ($p<0.05$) lower GAI compared to all the other groups.

The data for gastric emptying is presented in **Fig 7.4**, the induction of diabetes accelerated gastric emptying significantly ($p<0.05$), while NC, DX and DA groups where able to significantly ($p<0.05$) reduce the gastric emptying at a similar capacity compared to the DC group. There was no significant difference between the DE group and all the other groups, however it was relatively higher compared to NC, DX and DA groups.

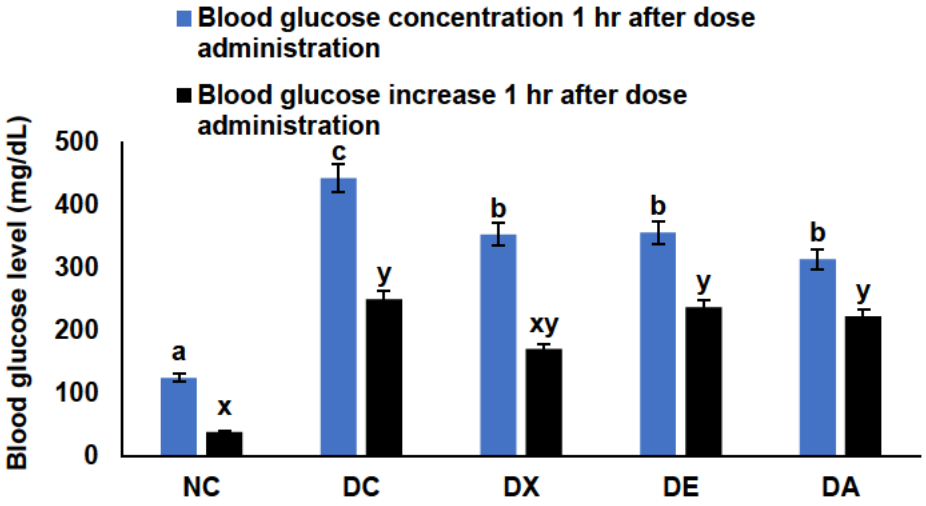

Figure 7.2: Effects of xylitol and erythritol on final glucose concentrations and blood glucose increase in different animal groups after 1 h of experimental period. Values = mean ± SD; n = 6 (NC, DC, DX, DE and DA).[a-d & x-y]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX = Diabetic xylitol, DE =Diabetic erythritol and DA =Diabetic acarbose

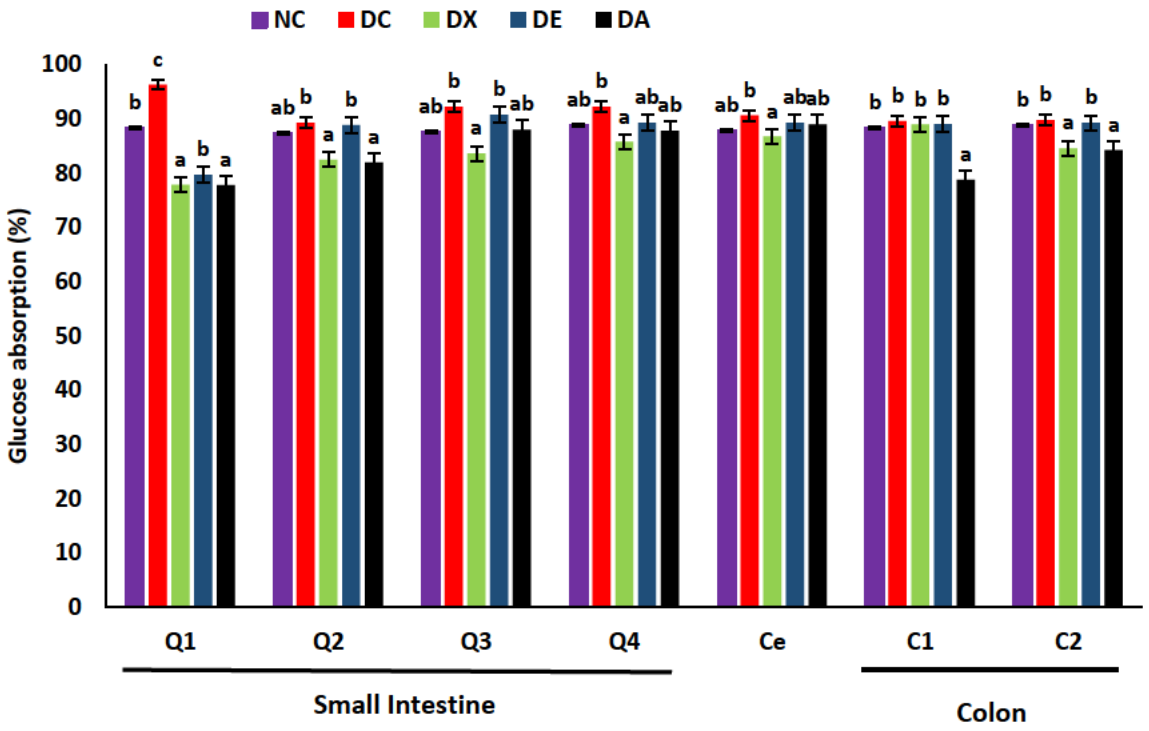

Figure 7.3: **Glucose absorption index (GAI) in the different GIT segments of different animal groups at the end of a 1-h experimental period**. Values = mean ± SD; n = 6 (NC, DC, DX, DE and DA).[a-d]Different letters presented above the bars for a given segment of gastrointestinal tract are significantly different from each other ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX = Diabetic xylitol, DE =Diabetic erythritol and DA =Diabetic acarbose

The data for the percentage of digesta transit in the different segments of GIT of all groups are presented in **Table 7.1**. The digesta transit for all groups was decreased from the small intestine to the proximal colon. Although no significant difference of digesta transit was observed in the first, second and third quarter of small intestine, cecum and proximal colon among the animal groups, significantly ($p<0.05$) lower digesta transit was observed in the last quarter of the small intestine of DE group compared to DX group, when no significant difference was observed among other groups.

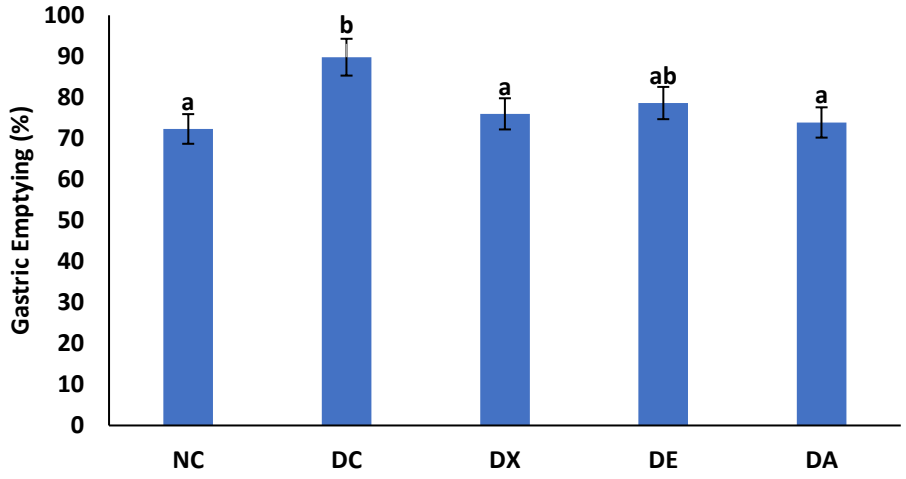

Figure 7.4: Effects of xylitol and erythritol on gastric emptying in different animal groups after 1 h experimental period. Values = mean ± SD; n = 6 (NC, DC, DX, DE and DA).[a-b]Different letters presented above the bars are significantly different from each other group ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX = Diabetic xylitol, DE =Diabetic erythritol and DA =Diabetic acarbose

Table 7.1: Data showing the percentage of digesta transit in the different segments of the GIT during 1 hour of experimental period.

	Small Intestine					Colon
Group	**Q1**	**Q2**	**Q3**	**Q4**	**Ce**	**Proximal**
NC	86.55±6.42	84.48±5.86	78.10±7.38	70.93±5.13[ab]	64.83±12.78	48.48±9.63
DC	84.08±4.02	83.64±3.61	76.79±4.90	65.79±6.16[ab]	56.42±9.11	46.56±4.63
DX	86.71±2.86	82.40±2.12	77.59±4.45	76.77±8.828[b]	54.84±6.25	49.75±6.29
DE	86.00±2.86	82.40±4.29	73.45±6.93	63.89±10.56[a]	54.49±9.11	49.56±5.46
DA	83.45±9.17	78.31±12.54	77.83±12.20	66.23±16.61[ab]	53.74±15.60	48.34±13.76

Values = mean ± SD; n = 6 (NC, DC, DX, DE and DA).[a-b]Different letters presented within a column for a given segment of gastrointestinal tract are significantly different from each other ($p < 0.05$). Tukey's HSD post-hoc test, SPSS, version 25, IBM, USA). NC = Normal control, DC = Diabetic control, DX = Diabetic xylitol, DE =Diabetic erythritol and DA =Diabetic acarbose.

7.5 Discussion

Sugar alcohols have been used as supplements in various foods and food products (Malgorzata Grembecka, 2018). Studies have reported that they may be effective in ameliorating diabetes, obesity and metabolic syndrome related abnormalities (Wolever *et al.*, 2002; Islam, 2011). In previous studies, it has been speculated that xylitol and erythritol have the ability to inhibit intestinal glucose absorption (Chukwuma and Islam, 2015; Chukwuma *et al.*, 2018). However, comparative effects of xylitol and erythritol on intestinal glucose absorption has not been investigated. Hence, the present study comparatively investigated the effects of xylitol and erythritol on intestinal glucose absorption in experimentally induced T2D model of rats.

Postprandial glucose is mostly absorbed from the proximal section of small intestine, however absorption capacity differs in the different segments of the small intestine (Weber and Ehrlein, 1998). A previous study revealed that glucose absorption was highest at the duodenum and lowest at the ileum in albino rats (Oluwasogo *et al.*, 2016). In another study, the highest glucose absorption capacity was observed in the distal ileum of rats (Sanford and Smyth, 1974). In the present study, although lower glucose absorption was observed for DE group only in the first and last quarter of the small intestine compared to DC group, the glucose absorption index for xylitol fed or DX group was significantly lower in the entire small intestine which is even better than acarbose fed or DA group in overall situation. Our results for the glucose absorption index for xylitol fed group are also consistent with previously published studies (Fearon and Bird, 1968; Chukwuma and Islam, 2015). This indicates the greater potential of xylitol in inhibiting intestinal glucose absorption compared to erythritol in type 2 diabetic condition.

Glucose is rapidly absorbed from the small intestine in both normal and diabetic states (Basu *et al.*, 2001; Holst *et al.*, 2016), therefor, the normal and diabetic animals presented similar glucose absorption indexes in terms of the location of gastrointestinal tracts (**Fig. 7.3**). Although the normal animals display glucose homeostasis, it is impaired in diabetic animals (Aronoff *et al.*, 2004), which is shown by the increased blood glucose level in the DC group compared to NC group in our study (**Fig. 7.2**). In our study, the relatively higher reduction of blood glucose level in xylitol fed animal group compared to the animals treated with erythritol (**Fig. 7.2**) could be due to the lower intestinal glucose absorption in the DX group compared to the DE group (**Fig. 7.3**). This data implies the greater potency of xylitol compared to erythritol in ameliorating hyperglycemia. A previous study has also reported the inhibitory effect of xylitol on intestinal glucose absorption (Chukwuma and Islam, 2015). However, data from our current study showed the better potency of xylitol in lower intestinal glucose absorption as well as lower blood glucose level compared to erythritol.

Gastric emptying and gastrointestinal transit time may be important determinants of glucose homeostasis and metabolic health through effects on nutrient digestion and absorption (Van Weyenberg *et al*., 2006; Hellström *et al*., 2006). It has been reported that delayed gastric emptying and accelerated digesta transit can cause delayed intestinal nutrient absorption and reduced food intake (Shafer *et al.*, 1987; Salminen *et al.*, 1989). In the present study, the DC group significantly increased gastric emptying which correlates with the lower phenol red distribution in the stomach compared to the NC group (**Fig. 7.1** and **7.4**). It has been reported that type 2 diabetic patients exhibit rapid gastric emptying which contributes to worsening of their glucose homeostasis (Phillips *et al*., 1992). The animals treated with xylitol had relatively slower gastric emptying which corresponds to the higher phenol red concentration in the stomach when compared to the animals treated with erythritol (**Fig. 7.1 and 7.4**). The slower or delayed gastric emptying as observed in the DX group could be a factor that

contributed to decline the intestinal glucose absorption of this group. Additionally, the xylitol treated animals also displayed a relatively faster digesta transit in the small intestine when compared the DE group (**Table 7.1**). This observation can also contribute to the reduced glucose absorption from the small intestine of DX group and ultimately reduced the blood glucose level in xylitol fed group compared to the erythritol group. These observations again imply the better hypoglycemic and antidiabetic potential of xylitol compared to erythritol.

7.6 Conclusion

The data from this study suggests that xylitol exhibits better hypoglycemic and antidiabetic effects by not only delaying gastric emptying as well as by increasing intestinal transit time but also by reducing small intestinal glucose absorption compared to erythritol. However, further clinical studies are required to ascertain the findings of this study.

CHAPTER 8

General discussion and conclusion

8.1 General discussion

The increasing prevalence of diabetes mellitus (DM) is one of the largest global public health concerns (Al-Lawati, 2017; Liu *et al.*, 2020). It has become a global epidermic with a prevalence of 463 million reported in 2019, representing an 8.9% increase from the 2017 estimate of 425 million (IDF, 2019b). The projection for 2045 is estimated at 700 million which portrays a 51% increase from the current figure (463 million). The African region has the highest percentage increase of 143% (IDF, 2019b). This projection will exacerbate socioeconomic problems in the continent, contemplating the immense healthcare expenditure for diabetic people and the increased mortality from DM (Mutyambizi *et al.*, 2019; Mapa-Tassou *et al.*, 2019). Dietary habits and sedentary lifestyle are the major factors for the rapid increase in the prevalence of T2D and its associated complications among developing countries (Sami *et al.*, 2017).

The consumption of sugar or sugar-sweetened products have been a major factor in the development of obesity, metabolic syndrome and T2D in African countries (Manyema *et al.*, 2015; Audain *et al*., 2019). Nutritional guidelines to reduce consumption of added sugars to reduce the daily energy intake as well as to reduce the risk of diabetes has been implemented (IDF, 2014). Non-nutritional sweeteners have gained popularity as sugar replacers in various foods and drinks due to their significantly higher sweetness level compared to traditional sweeteners but also due to their low caloric values (Liauchonak *et al.*, 2019). However, various studies have reported the concerns regarding their association with increased risk to develop obesity, metabolic syndrome, and T2D (Ruanpeng *et al.*, 2017; Daher, Matta and Abdel Nour, 2019). There are many other controversial reports available regarding the safety

and toxicity of non-nutritive artificial sweeteners (Sharma *et al.*, 2016; Choudhary and Pretorius, 2017). In light of this, sugar substitutes such as sugar alcohols, including xylitol, sorbitol, mannitol, maltitol, lactitol, isomalt and erythritol, have brought an escalating interest in the management of diabetes (Wolever *et al.*, 2002; Grembecka, 2015; Msomi *et al*., 2021). Due to their lower calorie content compared to table sugar and antioxidative, antihyperglycemic and antidiabetic potentials (Awuchi, 2017; Grembecka, 2018; Wölnerhanssen *et al.*, 2019;Ibrahim, 2019), the use of sugar alcohols in various foods, drinks and food products is rapidly increasing all over the world (Grembecka, 2015b). Among these sugar alcohols, xylitol and erythritol have been extensively studied as sugar alternatives for diabetes due to their diabetes associated health benefits (Wölnerhanssen *et al.*, 2019), apart from many other beneficial effects on health. However, there is a dearth in comparative research on xylitol and erythritol on their antidiabetic efficacy. Hence, this study comparatively conducted the antioxidant, antihyperglycemic and antidiabetic effects as well as the mechanism of actions of xylitol and erythritol using *in vitro*, *ex vivo* and *in vivo* experimental models.

In *in vitro* condition (**Chapter 3**), xylitol and erythritol were able to dose dependently inhibit the key enzymes linked to T2D, with xylitol exhibiting the better inhibitory activity compared to erythritol. Accordingly, although both of these sugar alcohols were able to portray their antidiabetic potential by inhibiting intestinal glucose absorption as well as stimulating muscle glucose uptake *ex vivo* (**Chapter 3**), the results were better for xylitol compared erythritol treatment. Taken together the results of *in vitro* and *ex vivo* studies, xylitol has been found as a preferrable sugar alcohol over erythritol in terms of its antioxidative, antihyperglycemic and antidiabetic activities.

The antidiabetic properties of the sugar alcohols were further confirmed *in vivo* (**Chapter 4**) by their ability to deplete blood glucose level, increase serum insulin, improving pancreatic

β-cell function and glucose tolerance level. These actions can be attributed to the ability of xylitol and erythritol to inhibit the activities of glucose-6-phosphatase, fructose-1,6-bisphosphatase, glycogen phosphorylase, α-amylase, ATPase, acetylcholinesterase, with concomitant elevation of ENTPDase activity (**Chapter 5 and 6**). Both sugar alcohols led to improved pancreatic, hepatic and muscular morphology. The result from the *in vivo* studies suggests that xylitol at 10% dose was more effective in eliciting the antidiabetic property when compared to the other treatment groups with lower or higher concentrations (**Table 8.1**). Additionally, in order to understand the comparative effects of xylitol and erythritol on gastric emptying, digesta transit, intestinal glucose absorption and blood glucose level, a separate *in vivo* study was conducted in experimentally-induced T2D model of rats. In this study, xylitol (1 g/kg bw) showed better hypoglycemic and antidiabetic effect compared to erythritol (1 g/kg bw) by reducing intestinal glucose and postprandial blood glucose increase, while delaying gastric emptying and increased digesta transit (**Chapter 7**). The promising antidiabetic potential of xylitol identified in this study has been reported in previous studies (Rahman and Islam, 2014)(Islam and Indrajit, 2012).The capability of xylitol and erythritol to attenuate dyslipidemia revealed their antilipemic effect by reducing total cholesterol, LDL-cholesterol, triglycerides and lipase enzyme activity with concomitantly elevating HDL-cholesterol level (**Chapter 4 and 5**). The cardio, nephron and hepato – toxicity biomarkers which include ALT, AST, CK-MB, creatinine, urea and uric acid were depleted in both xylitol and erythritol treated animals (**Chapter 4**). In most of these parameter's 10% xylitol had the better effects compared to the other treatment groups of xylitol and erythritol. These results suggest that xylitol has potentially better ability to protect against diabetes and its associated complications compared to erythritol.

Xylitol also showed better oxidative stress alleviating potential than erythritol, by supressing DPPH, NO^- and FRAP activities, *in vitro* (**Chapter 3**). Accordingly, xylitol was able to

elevate the GSH, GPx and GR levels, SOD and catalase activities, with concomitant depletion of MDA levels in oxidative muscular injury at a greater capacity, *ex vivo* (**Chapter 3**). The antioxidative activity of the sugar alcohols were further confirmed *in vivo* by their ability to modulate redox imbalance in the serum, pancreatic, hepatic and muscular tissues (**Chapter 4-6**). Xylitol at 10% showed relatively better efficacy in ameliorating oxidative stress compared to the other treatment groups (**Table 8.1**). This observation further validates the higher potency of xylitol as an antioxidant which confirms an earlier report on its ability to ameliorate diabetes-induced oxidative stress (Kang *et al.*, 2007).

8.2 Conclusion

In summary, the overall beneficial effects of xylitol and erythritol revealed their antioxidant capacity, ability to inhibit carbohydrate digestive enzymes, lower blood glucose levels, stimulate insulin secretion, inhibit intestinal glucose absorption, enhance glucose uptake, and mitigate lipid dysmetabolism. In order to pin-point the comparative antioxidative (AO) and antidiabetic (AD) effects of xylitol and erythritol, a scoring table has been created as shown below (**Table 8.1**). Any significantly beneficial effect of xylitol or erythritol compared to diabetic control group were given +1 (p<0.05) or +2 (p<0.01) or +3 (p<0.001). At the end, total scores for the AO and AD effects of the individual dose of xylitol and erythritol were calculated including the means of AO and AD effects (**Table 8.1**). Based on the mean scores for each dose of xylitol and erythritol, 10% xylitol dose received the highest score followed by 10% erythritol, 20% xylitol, 20% erythritol, 5% erythritol and 5% xylitol.

Table 8.1: Summary of the scoring values *in vivo* for the antidiabetic and antioxidative effects of the tested sugar alcohols

	DX5		DX10		DX20		DE5		DE10		DE20	
	AD	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**
Scores from *in vivo* study 1 (Chapter 4)	+8	+15	+27	+24	+18	+24	+16	+21	+22	+24	+20	+24
Scores from *in vivo* study 2 (Chapter 5)	+13	+11	+16	+12	+13	+11	+13	+11	+15	+12	+13	+11
Scores from *in vivo* study 3 (Chapter 6)	+16	+10	+24	+12	+22	+11	+16	+10	+21	+11	+18	+11
Final score	+37	+36	+67	+48	+53	+46	+45	+43	+58	+47	+51	+46
Average score	**+36.5**		**+57.5**		**+49.5**		**+44**		**+52.5**		**+48.5**	

Notes: Values were adapted from supplementary data 1-3. NC = Normal control, DC = Diabetic control, DX 5 = Diabetic xylitol 5%, DX 10 = Diabetic xylitol 10%, DX 20 = Diabetic xylitol 20%, DE 5 = Diabetic erythritol 5%, DE 10 = Diabetic erythritol 10% ,DE 20 = Diabetic erythritol 20%, AD = Antidiabetic and AO = Antioxidative.

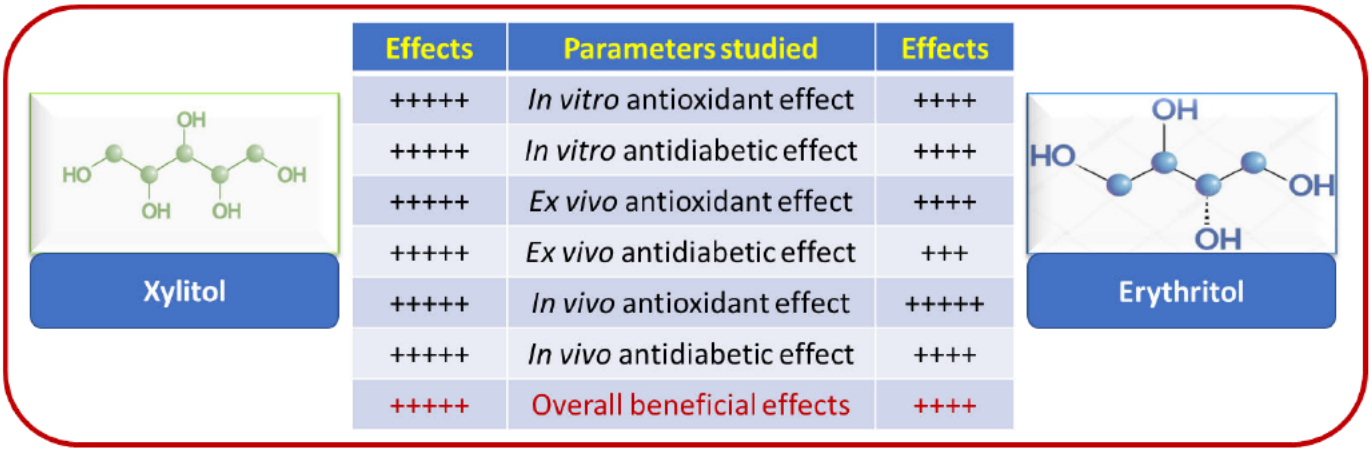

Figure 8.1 Schematic diagram showing the overall comparative antioxidant and antidiabetic beneficial effects of xylitol and erythritol. Positive (+) sign compares the significant antioxidant and antidiabetic effects of the treatment groups.

8.3 Recommendations

Considering all above, it can be suggested that xylitol holds the most beneficial antioxidant and antidiabetic potency compared to erythritol, when 10% xylitol has been found as most beneficial dose compared to any dosages of xylitol or erythritol used in this study. Hence, xylitol can be used as a preferrable alternative sweetener or supplements over erythritol in the diabetic foods and food products for the management of diabetes and its associated complications. However, further clinical studies are required in order to ascertain the results of this study.

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APPENDIX 1

ANIMAL ETHICS APPROVAL

BIOMEDICAL RESOURCE UNIT

June 19, 2017

Dear Prof Islam
Chair: Animal Research Ethics Committee
c/o School of Life Sciences

RE: ATTENDANCE OF LAS COURSE

This letter certifies that Miss Nontokozo Msomi have attended the Laboratory Animal Course that was hosted by the Biomedical Resource Unit.

The course was held on the 11 – 12 May 2017 and entailed the following:

Introduction to laboratory animal sciences. Bioethics and Animal experimentation. Animal Research Methodology. Experimental design, environmental enrichment and occupational safety.

The course was completed satisfactorily and may be allowed to initiate her research after the relevant practical procedures was done to a level of competency that was signed off by the veterinarian in charge.

Kind Regards

Dr SD Singh BVSc. (Mumbai) MS (Illinois) LAS (Utrecht) CVE (Pretoria)
HOD: Biomedical Resource Unit
Veterinarian

Miss Ritta Radebe, Room 201, 2nd Floor U- Block ,Tel 031 260 7671,Fax 031 260 7730 E-mail radeber@ukzn.ac.za

REDUCE, REFINE AND REPLACE

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

07 September 2020

Ms Nontokozo Zimbili Msomi (215075542)
School of Life Sciences
Westville Campus

Dear Ms Msomi,

Protocol reference number: AREC/037/019D
Project title: Antioxidant and anti-diabetic effects of Xylitol and Erythritol: A comparative study.

Full Approval – Research Application

With regard to your revised application received on 17 August 2020, the Animal Research Ethics Committee has accepted the documents submitted and **FULL APPROVAL** for the protocol has been granted.

Please note: There must be adherence to national and institutional COVID-19 regulations and guidelines at all times. Researchers will be personally responsible and liable for non-adherence to national regulations. If in doubt, please contact the Research Ethics Chair and/or the University Dean of Research for advice.

Please note: Any Veterinary and Para-Veterinary procedures must be conducted by a SAVC registered VET or SAVC authorized person.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 06 September 2021.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

.....
Dr Sanil D Singh, PhD
Chair: Animal Research Ethics Committee

/kr
cc Supervisor: Prof S Islam
cc BRU Manager: Dr Jaca

Animal Research Ethics Committee (AREC)
Ms Karen Reinertsen (Administrator)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
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APPENDIX 2

PUBLICATIONS FROM THIS THESIS

REVIEW ARTICLE

Suitability of sugar alcohols as antidiabetic supplements: A review

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[b] Department of Pharmacology, School of Clinical Medicine, Faculty of Health Sciences, University of the Free State, Bloemfontein 9300, South Africa

Abstract

The major goals in the management of diabetes are to maintain optimum control of high blood glucose level or hyperglycemia. Dietary modification is one of the most recommended treatment modalities for diabetic patients. The use of foods sweetened with sugar alcohols (also known as polyols) such as xylitol, sorbitol, mannitol, maltitol, lactitol, isomalt and erythritol has brought an escalating interest in the recent years since some sugar alcohols do not rise plasma glucose, as they are partially digested and metabolised. Diet composition and adequacy may be altered by replacing carbohydrates with sugar alcohols. It has been established that these polyols are appropriate sugar substitutes for a healthy lifestyle and diabetic foods. The present review focuses on the evidence supporting the use of sugar alcohols in the management of diabetes, by evaluating their physical and chemical properties, metabolism, absorption, glycemic and insulinemic responses. Although documentation on the glycaemic and insulinemic response of polyols is evident that these compounds have beneficial effects on the better management of hyperglycemia, the possible side effects associated with their normal or higher dosages warned their use according to the relevant Food & Drug Administration guidelines. For the same reason, future studies should also focus on the possible toxicity and side effects associated with the consumption of sugar alcohols in order to define their safety.

Keywords: Diabetes, Glycemic index, Insulinemic index, Sugar alcohols, Sweeteners

1. Introduction

The global prevalence of diabetes is increasing progressively, with an estimated 463 million people living with diabetes and a projection of 700 million by 2045, the vast majority of whom have type 2 diabetes (T2D) [1]. Epidemic of diabetes has astounding healthcare expenditure and significant impacts in the quality of life [2]. In 2019, the global diabetes-related healthcare expenditure reached an estimated 760 billion USD, with a 14.5% increase from 2017 [1].

Diabetes is a multifactorial disease and one of its most prominent association is the consumption of excess refined sugar or sugar containing foods and food products [3,4]. Therefore, the World Health Organization issued nutritional guidelines not only to reduce the consumption of added sugars but also to reduce the daily energy intake from refined sugar [5]. This has led to the identification of modifiable diets and lifestyle factors for the prevention and better management of diabetes [6,7]. A growing interest in products sweetened with sugar alcohols in the market has transpired, targeted towards people with diabetes [8,9]. Studies have suggested that sugar alcohols have the ability to alter the nutritional adequacy of a diet since they have lower energy values due to the way they are metabolized [10]. Therefore, these sugar substitutes are useful in the maintenance of a nutritionally balanced diet for diabetic individuals [11]. Considering all above, the present review was designed to analyse the beneficial effects, mechanisms of actions and possible side effects of different widely used sugar alcohols for the better and safer management of hyperglycemia and diabetes.

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APPENDIX 3

OTHER PUBLICATIONS AND SUBMITTED ARTICLES

Food and Chemical Toxicology 154 (2021) 112335

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Cola nitida infusion modulates cardiometabolic activities linked to cardiomyopathy in diabetic rats

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ARTICLE INFO

Handling Editor: Dr. Jose Luis Domingo

Keywords:
Cardiometabolism
Cardiomyopathy
Cola nitida
Diabetes mellitus

ABSTRACT

This study investigated the therapeutic mechanism of *Cola nitida* seeds on diabetic cardiomyopathy in hearts of diabetic rats. Type 2 diabetic (T2D) rats were treated with *C. nitida* infusion at 150 or 300 mg/kg body weight (bw). The rats were sacrificed after 6 weeks of treatment, and their hearts harvested. There was an upsurge in oxidative stress on induction of T2D as depicted by the depleted levels of glutathione, superoxide dismutase and catalase activities, and elevated malondialdehyde level. The activities of acetylcholinesterase, and ATPase were significantly elevated, with suppressed ENTPDase and 5'nucleotodase activities in hearts of T2D rats depicting cholinergic and purinergic dysfunctions. Induction of T2D further led to elevated activity of ACE and altered myocardial morphology. Treatment with *C. nitida* infusion led to reversal of these biomarkers' activities and levels, while maintaining an intact morphology. The infusion caused decreased lipase activity and depletion of diabetes-generated cardiac lipid metabolites, while concomitantly generating saturated and unsaturated fatty acids, fatty esters and alcohols. There was also an inactivation of plasmalogen synthesis and mitochondrial beta-oxidation of long chain saturated fatty acids pathways in T2D rats treated with *C. nitida* infusion. These results indicate the therapeutic effect of *C. nitida* infusion against diabetic cardiomyopathy.

1. Introduction

Diabetes mellitus (DM) is regarded as a metabolic disease as it affects the metabolism of carbohydrate, protein and lipid. It is mainly characterized by inability of the pancreatic β-cell to secrete insulin and/or inability of the body to utilize secreted insulin, thereby leading to increased blood glucose level (hyperglycemia). The former is the main characteristics of type 1 diabetes (T1D), while the latter is characteristic of type 2 diabetes (T2D), the most prevalent amongst the diabetes types (I.D.F., 2016; IDF, 2019). Chronic hyperglycemia arising from insulin resistance and β-cell dysfunction has been reported as the main culprit of T2D and responsible for the macrovascular and microvascular complications associated with the disease (Aronson, 2008).

Cardiomyopathy is among the complications of both T1D and T2D resulting to heart failure. This is evidenced by studies linking DM with diastolic dysfunction characterized by exacerbated interstitial and perivascular fibrosis, and an enlarged left ventricle leading to an abnormal cardiac morphology (Borghetti et al., 2018; Fang et al., 2004). Oxidative stress and lipotoxicity arising from chronic hyperglycemia and lipid dysmetabolism respectively have been reported among the pathologic mechanisms of diabetic cardiomyopathy (Hamblin et al., 2007; Ussher, 2014; van de Weijer et al., 2011). Altered energy production and increased levels of angiotensin II have also been implicated in the pathogenesis and progression of diabetic cardiomyopathy (Frustaci et al., 2000; Oudit et al., 2007).

Medicinal plants have been employed in the treatment of DM and its complications in folk medicine from ancient times. The antidiabetic efficacies of some of these plants have been reported in both pre-clinical and clinical studies (Chukwuma et al., 2019; Mohammed et al., 2014). *Cola nitida* ranks amongst these medicinal plants and its antidiabetic activity has been established in T2D rats (Dorathy et al., 2014; Erukainure et al., 2019b). Its seeds are commonly referred as kola nut and indigenous to the West African countries, Nigeria, Ghana, Benin, Liberia, Cote D'Ivoire and Sierra Leone (Burdock et al., 2009). It is used for food, as well as social, religious and ceremonial activities (Asogwa et al.,

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The antioxidant and antidiabetic potentials of polyphenolic-rich extracts of Cyperus rotundus (Linn.): In vitro and in silico studies

Journal:	*Journal of Biomolecular Structure & Dynamics*
Manuscript ID	TBSD-2021-0861
Manuscript Type:	Research Article
Date Submitted by the Author:	09-May-2021
Complete List of Authors:	Mohamed, Almahi; University of KwaZulu-Natal, Department of Biochemistry Besani, Brian; University of KwaZulu-Natal, Department of Biochemistry Msomi, Nontokozo; University of KwaZulu-Natal, Department of Biochemistry Salau, Veronica; University of KwaZulu-Natal, Department of Biochemistry Erukainure, Ochuko; University of the Free State, Department of Pharmacology Islam, Shahidul; University of KwaZulu-Natal, Department of Biochemistry
Keywords:	Antioxidant, Antidiabetic, Cyperus rotundus L., Molecular Docking

Journal of
Food Biochemistry

Xylitol has better carbohydrate digesting enzymes inhibitory and antioxidant activities compared to erythritol in vitro: A comparative study

Journal:	*Journal of Food Biochemistry*
Manuscript ID	JFBC-08-20-1308
Manuscript Type:	Short Communication
Date Submitted by the Author:	10-Aug-2020
Complete List of Authors:	Msomi, Nontokozo; University of KwaZulu-Natal - Westville Campus, Islam, Shahidul; University of KwaZulu-Natal - Westville Campus, Discipline of Biochemistry
Keywords:	Digestive enzymes, Antioxidants, Sugar Alcohols, Xylitol, Erythritol, Diabetes

APPENDIX 4

SUPPLEMENTARY DATA

Supplementary data 1: The scoring values from *in vivo* study (Chapter 4) for the antidiabetic and antioxidative effects of the tested sugar alcohols

	DX5		DX10		DX20		DE5		DE10		DE20	
Parameters	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**
Fluid intake	+3		+3		+3		+3		+3		+3	
Food intake	+3		+3		+3		+3		+3		+3	
Pancreatic weight	0		0		0		0		0		0	
Blood glucose (Week 8)	0		+3		+1		+1		+2		+1	
Serum insulin	0		+2		0		0		0		0	
HOMA-IR	0		+3		+3		+3		+3		+3	
HOMA - β	0		+3		0		0		+3		+3	
Total-Chol	0		+1		0		0		0		+1	
HDL-Chol	0		0		0		0		0		0	
LDL-Chol	0		+3		+1		+1		+2		+2	
Triglycerides	0		+2		+2		+1		+2		+2	
ALT	+2		+2		+3		+2		+2		+2	
AST	0		+1		+2		+2		+1		0	
CK-MB	0		+1		0		0		0		0	
Creatinine	0		0		0		0		+1		0	
Urea	0		0		0		0		0		0	
Uric acid	0		0		0		0		0		0	
GSH Pancreas		0		+3		+3		0		+3		+3
GSH Serum		0		+3		+3		+3		+3		+3
MDA Pancreas		+3		+3		+3		+3		+3		+3
MDA Serum		0		+3		+3		+3		+3		+3
SOD Pancreas		+3		+3		+3		+3		+3		+3
SOD Serum		+3		+3		+3		+3		+3		+3
CAT Pancreas		+3		+3		+3		+3		+3		+3
CAT Serum		+3		+3		+3		+3		+3		+3
Score in each Parameter	+8	+15	+27	+24	+18	+24	+16	+21	+22	+24	+20	+24
Average Score	+11.5		+25.5		+21		18.5		23		22	

Notes: Values 1–3 means first to third level (1–3) of significant difference ($p < 0.05$) of treatment groups compared to the diabetic control groups. NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol

5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 = Diabetic erythritol 5%, DE10 = Diabetic erythritol 10% ,DE20 = Diabetic erythritol 20%, AD = Antidiabetic and AO = Antioxidative.

Supplementary data 2: The scoring values from *in vivo* study (Chapter 5) for the antidiabetic and antioxidative effects of the tested sugar alcohols

	DX5		DX10		DX20		DE5		DE10		DE20	
Parameters	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**
Liver weights	+1		+1		+1		+1		+1		+1	
Relative liver weights	0		0		0		0		0		0	
Glycogen Content	0		+2		0		0		0		+1	
ATPase activity	+3		+3		+3		+3		+3		+3	
ENTPDase activity	+3		+3		+3		+3		+3		+3	
Acetylcholinesterase	+1		+2		+1		+1		+1		+1	
Lipase	+2		+2		+1		+1		+2		+2	
Total-Chol	+3		+3		+3		+3		+3		+2	
HDL-Chol	0		0		0		+1		0		0	
LDL-Chol	0		0		0		0		0		0	
Triglycerides	0		0		+1		0		0		0	
Glutathione		+3		+3		+3		+3		+3		+3
Malondialdehyde		+3		+3		+3		+3		+3		+3
Superoxide dismutase		+3		+3		+3		+3		+3		+3
Catalase		+2		+3		+2		+2		+3		+3
Score in each Parameter	+13	+11	+16	+12	+13	+11	+13	+11	+15	+12	+13	+11
Average Score	12		+14		+12		+12		+13.5		+12	

Notes: Values 1–3 means first to third level (1–3) of significant difference ($p < 0.05$) of treatment groups compared to the diabetic control groups. NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 = Diabetic erythritol 5%, DE10 = Diabetic erythritol 10% ,DE20 = Diabetic erythritol 20%, AD = Antidiabetic and AO = Antioxidative

Supplementary data 3: The scoring values from *in vivo* study (Chapter 6) for the antidiabetic and antioxidative effects of the tested sugar alcohols

	DX5		DX10		DX20		DE5		DE10		DE20	
Parameters	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**	**AD**	**AO**
ATPase activity	0		+3		+1		0		+1		0	
ENTPDase activity	+3		+3		+3		+3		+3		+3	
Acetylcholinesterase	+1		+3		+3		+1		+3		+2	
Lipase	0		+3		+3		0		+2		+1	
Glucose – 6 – phosphatase activity	+3		+3		+3		+3		+3		+3	
Fructose – 1,6 – bisphosphatase activity	+3		+3		+3		+3		+3		+3	
Glycogen phosphorylase activity	+3		+3		+3		+3		+3		+3	
Amylase activity	+3		+3		+3		+3		+3		+3	
Glutathione		+3		+3		+3		+3		+3		+3
Malondialdehyde		+3		+3		+3		+3		+3		+3
Superoxide dismutase		+3		+3		+3		+3		+3		+3
Catalase		+1		+3		+2		+1		+2		+2
Score in each Parameter	+16	+10	+24	+12	+22	+11	+16	+10	+21	+11	+18	+11
Average Score	13		18		16.5		13		16		14.5	

Notes: Values 1–3 means first to third level (1–3) of significant difference ($p < 0.05$) of treatment groups compared to the diabetic control groups. NC = Normal control, DC = Diabetic control, DX5 = Diabetic xylitol 5%, DX10 = Diabetic xylitol 10%, DX20 = Diabetic xylitol 20%, DE5 = Diabetic erythritol 5%, DE10 = Diabetic erythritol 10% ,DE20 = Diabetic erythritol 20%, AD = Antidiabetic and AO = Antioxidative