Shelf-life studies on Sulphated Polysaccharides from some South African seaweeds and their protective effect against diabetic hepatopathy in rats with type-2 diabetes

Lethiwe Bashadile Mpungose

(218006861)

Submitted in fulfillment of the academic requirement of the Master of Science degree in the Discipline of Microbiology, School of Life Sciences, College of Agriculture Engineering and Science at the University of KwaZulu-Natal Westville Campus

JULY 2025

As the candidate's supervisor, I have approved the dissertation for submission.

Signed: [redacted] Name: Prof AO Olaniran Date: 17/07/2021

Signed: [redacted] Name: _Tosin A. Olasehinde Date: __17th July 2025___

PREFACE

The experiment work conducted and described in this dissertation was carried out in the Discipline of Microbiology, School of Life Sciences at the University of KwaZulu-Natal – Westville Campus, Durban, South Africa from May 2023-July 2025, under the supervision of Prof. A.O. Olaniran.

This study is an original representation of the work the author conducted and has not been submitted in any form for any degree or diploma to any tertiary institution. Usage of the work of others is fully acknowledged in the text in the most respected and accepted manner.

COLLEGE OF AGRICULTURE, ENGINNERING AND SCIENCE

DECLARATION 1 - PLAGIARISM

I, Lethiwe Bashadile Mpungose, declare that:

1. The research reported in this dissertation, except where otherwise stated, is my original research work.
2. The dissertation has not been submitted for any degree or examination at any other university.
3. This dissertation does not contain other scientists' data, pictures, graphs, or any other information, unless specifically acknowledged as being sourced from other scientists.
4. This dissertation does not contain other scientists' writings, unless otherwise acknowledged as being the sourced from other scientists. Where other written sources have been quoted, then their words have been re-written, but the general information attributed to them has been referenced.
5. This dissertation does not contain text, graphics or tables taken from the internet, unless specifically acknowledged, and the source being detailed in the dissertation in the reference section.

LETHIWE BASHADILE MPUNGOSE (Candidate)

COLLEGE OF AGRICULTURE, ENGINEERING, AND SCIENCE

DECLARATION 2- PUBLICATION

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this dissertation (include publication, submitted, in press and published and give details of the contributions of each author to the experimental work and writing of each publication).

Publication 1: Sulphated polysaccharides from *Ecklonia maxima* (Brown Seaweed) protects against diabetic hepatopathy by mitigating glucolipotoxicity and oxidative stress in hepatic tissues of type- 2 diabetic rats. (**In preparation**).

Publication 2: Sulphated polysaccharides from *Gracilaria gracilis* (Red Seaweed) mitigates diabetic hepatopathy by ameliorating glucolipotoxicity and amino acid dysmetabolism, while exacerbating antioxidative activities in hepatic tissues of type- 2 diabetic rats. (**In preparation**)

Publication 3: Influence of storage and temperature on the stability and antidiabetic properties of sulphated polysaccharides from Red (*Gracilaria gracilis*) and Brown (*Ecklonia maxima*) seaweeds. (**In preparation**).

LETHIWE BASHADILE MPUNGOSE (Candidate)

ACKNOWLEDGEMENTS

I have been carried gracefully by my Lord and Savior Jesus Christ; I could not fathom His grace and mercy towards me this entire period.

I am highly grateful and with great respect towards:

My supervisor, Prof. A.O. Olaniran, for his support, wisdom, and guidance throughout my research.

My co-supervisor Dr. T.A Olasehinde, and mentors, Dr. O.L. Erukainure, Dr A. Kumar, and Prof S. Islam for their time, guidance, assistance and support throughout my study.

To the Technology Innovation Agency, I am grateful for funding and supporting my study.

The staff members of the Biomedical Resource Unit (BRU), Dr. Linda Bester, Dr. Jaca and Mr. David for their support, assistance and guidance.

To the School of Life Sciences, Department of Microbiology staff members and lab 4 colleagues, thank you for lending a hand where necessary.

Above and beyond, Mrs. J.P. Mpungose (my mother) I appreciate you praying for me, supporting me emotionally, and the wisdom you imparted with me throughout this journey. My siblings, especially my brother (father), Mr. K.M Mpungose, and Ms. N.N. Mpungose (sister-mother) thank you for your endless support and for propelling me into greatness.

Table of Contents Pages

List of Figures **Pages**

CHAPTER ONE

CHAPTER THREE **PAGE**

LIST OF TABLES

temperature and time

ABSTRACT

Diabetic hepatopathy is among the major contributors to mortality and morbidities associated with type 2 diabetes (T2D). Sulphated polysaccharides (SPs) from seaweeds have antidiabetic potential, however, their effects on diabetic hepatopathy, a major diabetic complication is yet to be investigated. This study sought to investigate the protective potential of SPs from *Ecklonia maxima* and *Gracilaria gracilis* on diabetic hepatopathy of rats with T2D. Two groups of T2D rats were administered 150 and 300 mg/Kg bodyweight (bw) of *E. maxima* SPs, respectively. Another two groups administered similar doses for *G. gracilis* SPs. Water was administered to the T2D and negative control groups, while metformin served as the standard antidiabetic drug. Normal rats administered 300 mg/Kg bw SPs served as the toxicology group. The effect of storage temperature and time on the stability and antidiabetic properties of both SPs were investigated, by storing them at 4, 25 and 37 °C for 6 months. There was significant depletion in glutathione level, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase, while concomitantly exacerbating malondialdehyde level, fructose-1.6biphosphatase, glucose 6-phosphatase, glycogen phosphorylase, and acetylcholinesterase activities in hepatic tissues, on induction of T2D. Furthermore, T2D dysregulated glucose, lipid and amino acid metabolic pathways, and their metabolites. Treatment with SPs from *E. maxima* and *G. gracilis* significantly reversed the glutathione and malondialdehyde levels, and enzymes activities, while concomitantly upregulating metabolic pathways and their metabolites. In the stability study, there were no significant changes in the glucose and sulphate levels of SPs stored at 4 and 25 °C. However, these levels were significantly reduced in SPs stored at 37 °C at the 5th and 6th month. Storage at 4 and 25 °C, had no significant effect on the α-glucosidase and αamylase

inhibitory activities of the SPs throughout the storage period. However, there was a time dependent decline in the inhibitory activities of these enzymes at 37 °C. These results indicate the hepatoprotective effect of SPs from *E. maxima* and *G. gracilis* against diabetic hepatopathy in T2D rats. This is depicted by their ability to mitigate oxidative stress, inflammation and lipotoxicity, while improving glucose and amino acid metabolisms. Furthermore, the stability of the SPs and their antidiabetic activities may be temperature dependent, influenced by storage time.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1. Introduction

Diabetes ranks among the world's fastest growing epidemic affecting over 537 million people in 2021, with Africa contributing to 4.5% (IDF 2021). A 46% increase to 783 million has been predicted for 2045, with a 134% increase from 24 million to 55 million predicted for Africa (Figure 1.1) (IDF 2021). The anticipated increase in Africa is a significant worry, mainly due to inadequate healthcare facilities, economic strains, and rising cases of other non-communicable diseases (NCDs), such as malaria, HIV/AIDS, and tuberculosis. The upward trend is attributed to increased urbanization resulting from rural-urban migration, sedentary lifestyles with reduced physical activity, and an ageing population (Mphasha and Stubbendorff 2025). Although Africa has the lowest number of people living with diabetes, more than 54% of individuals with diabetes in the region remain undiagnosed. Challenges in gathering data combined with the scarcity of high-quality information imply that the actual figure might be significantly higher.

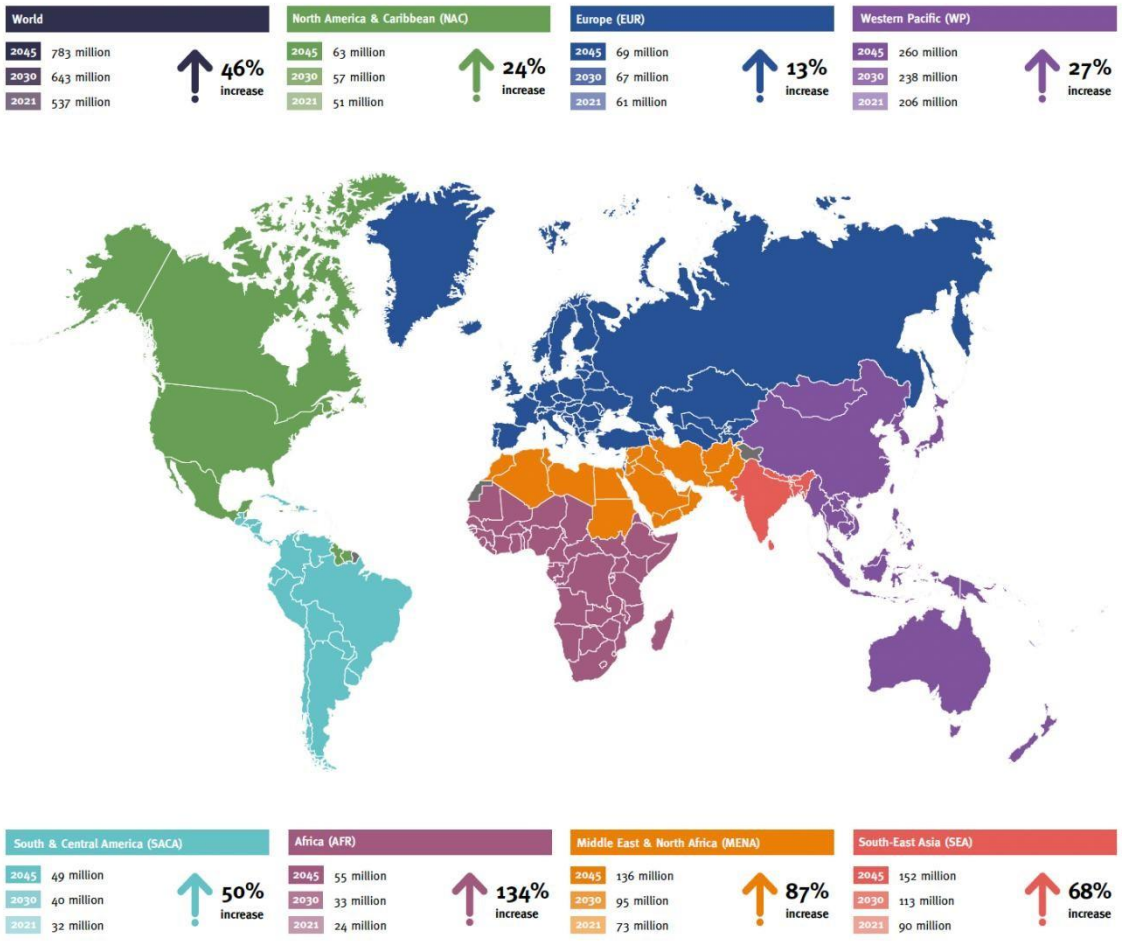


Figure 1.1. Number of people with diabetes worldwide and per International Diabetes Federation (IDF) Region in 2021–2045 (20–79 years). Adapted without permission from IDF Diabetes Atlas, 10th edition, 2021 (IDF 2021).

Diabetes is a major contributor to global mortality, with over 6.7 million deaths of adults between the age of 20–79 is being attributed to diabetes and its complications in 2021 (IDF 2021). This sums up to about 12.2% mortality rates from all deaths in this age group. About one-third of total deaths from diabetes occur in individuals under the age of 60, with 32.6% of these cases fitting this demographic **(Figure 1.2)**. This accounts for 11.8% of overall worldwide deaths among individuals under the age of 60.

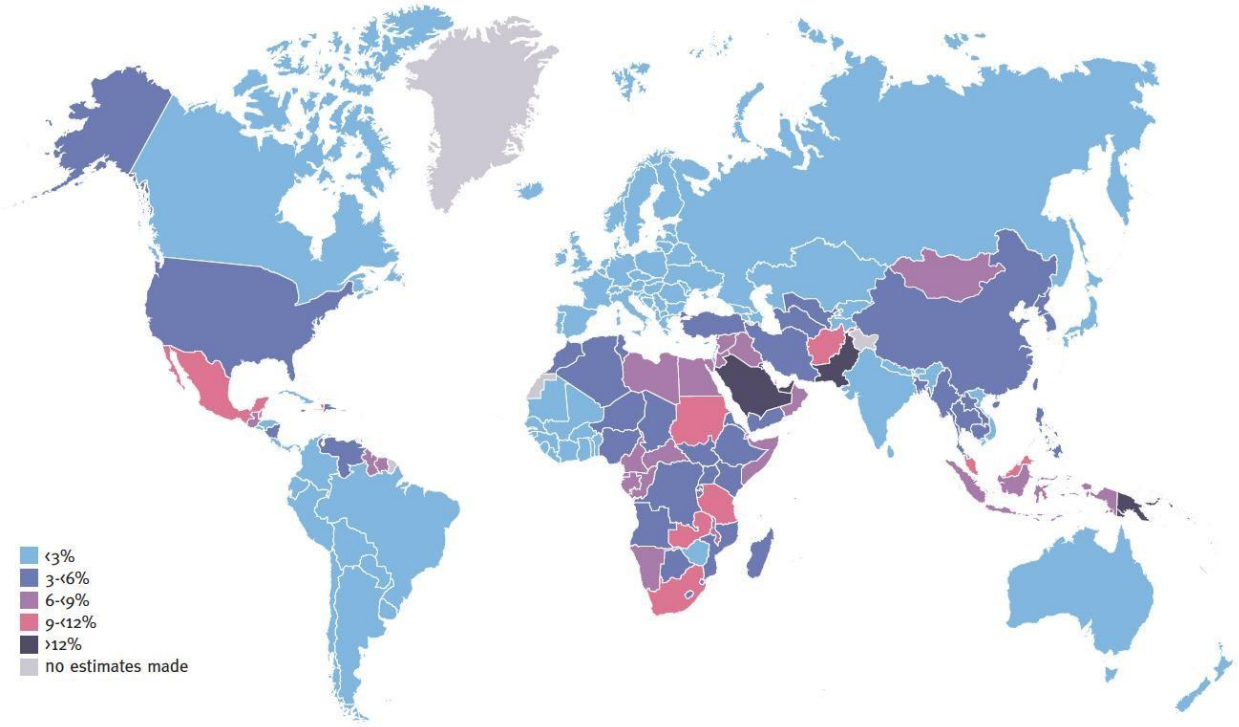


Figure 1.2. Number of diabetes-related deaths in adults (20–79 years), by age in 2021. Adapted without permission from IDF Diabetes Atlas, 10th edition, 2021 (IDF 2021).

The financial burden of diabetes and its complications is substantial, affecting individuals, their families, healthcare systems, and global economy (IDF 2021; Williams et al. 2020). In 2019, the global expenditure on diabetes was approximately USD 760 billion. This is anticipated to increase to USD 825 billion and USD 845 billion by 2030 and 2045, respectively (Williams et al. 2020). Annual health expenditures on diabetes show a considerable range of variation (Figure 1.3), with the US having the highest expenditure (USD 379.5 billion), followed by China (USD 165.3 billion) and Brazil (USD 42.9 billion) (IDF 2021). Africa has the lowest diabetes-related spending, estimated at USD 13 billion, which makes up 1% of worldwide diabetes-related expenditure (Mba and Mbanya 2020).

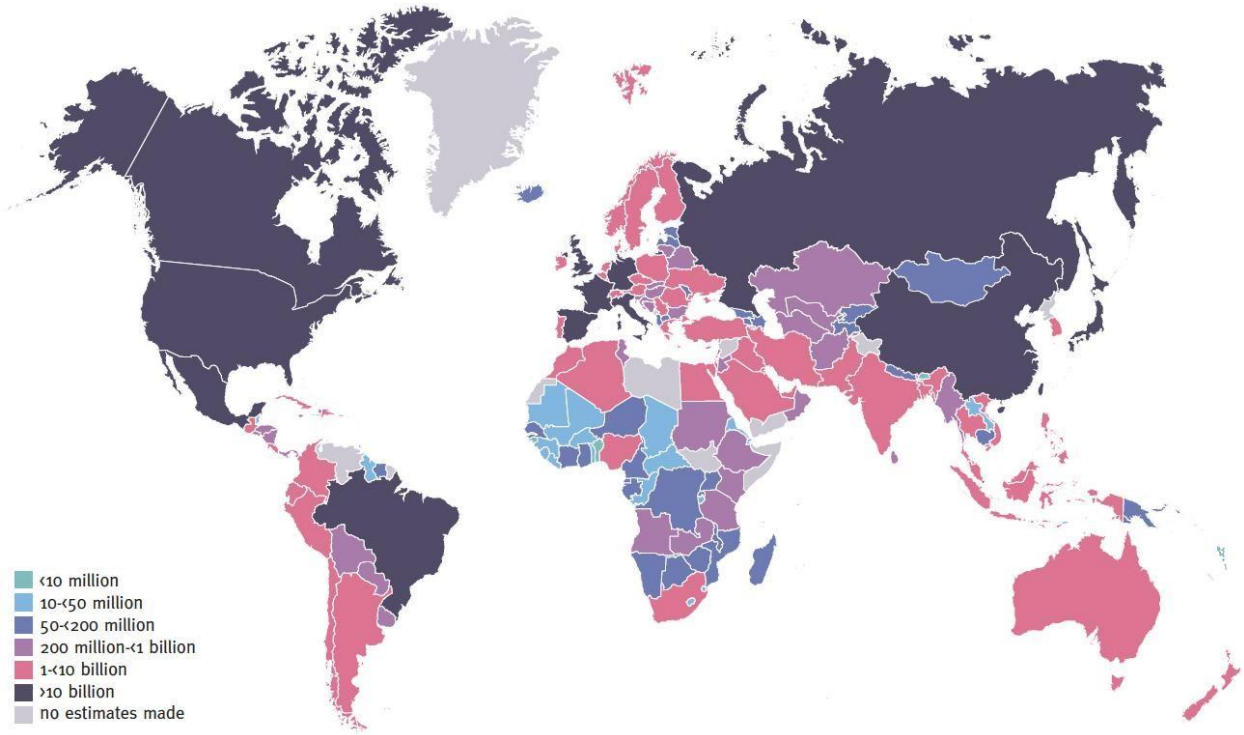


Figure 1.3. Total health expenditure (USD) for adults (20–79 years) with diabetes, in the year 2021. Adapted without permission from IDF Diabetes Atlas, 10th edition, 2021 (IDF 2021).

Owing to the disease burden and economic implications, there have been a continuous search for affordable alternative treatments, with a paradigm shift to natural products (Erukainure et al. 2018). Of these natural products, polysaccharides, especially sulphated polysaccharides (SPs) from seaweeds, have been gaining much interests on their therapeutic roles in the management of diabetes and its complications. Hence, this project was aimed at investigating the therapeutic effect of SPCs on diabetes and its complications, particularly hyperglycemia-mediated hepatopathy in type 2 diabetes (T2D).

1.1 Diabetes

Diabetes is a metabolic disease characterized by hyperglycemia (high blood glucose level) arising from defects in insulin secretion and/or action which affects carbohydrate, fat, and protein metabolism. The disease is associated with various underlying causes and mechanisms of

development involving pancreatic β-cells dysfunction induced by glucotoxicity, lipotoxicity, heightened inflammation and oxidative stress, and endoplasmic reticulum dysfunction (Cernea and Dobreanu 2013; Eguchi et al. 2021; Poitout and Robertson 2008; Vilas-Boas et al. 2021). These etiologies have been implicated in the pathogeneses of hyperglycemia-mediated complications such as cardiovascular diseases (CVD), retinopathy, neuropathy, nephropathy, and hepatopathy (Giri et al. 2018; Li et al. 2023).

Insulin is a polypeptide hormone produced by the pancreatic β-cells located within the islets of Langerhans and plays a primary role in regulating blood glucose levels (Qaid and Abdelrahman 2016; Wilcox 2005). As shown in Figure 1.4, It decreases blood glucose levels by stimulating its uptake into skeletal muscles and other insulin-sensitive tissues through the activation of glucose transporter 4 (GLUT4) transporters, which in turn triggers glycogenesis while simultaneously slowing down glycogenolysis (Dimitriadis et al. 2011). Insulin also reduces plasma fatty acid levels by promoting the uptake of fatty acids into both adipose and muscle tissues; while suppressing the breakdown of fatty acids in adipose tissue and their oxidation in muscle and liver (Carpentier 2021; Dimitriadis et al. 2011). Therefore, insulin deficiency, and/or underutilization have tremendous metabolic fate which has been implicated in the pathogenesis of diabetes and its complications (Antar et al. 2023; Petersen and Shulman 2018).

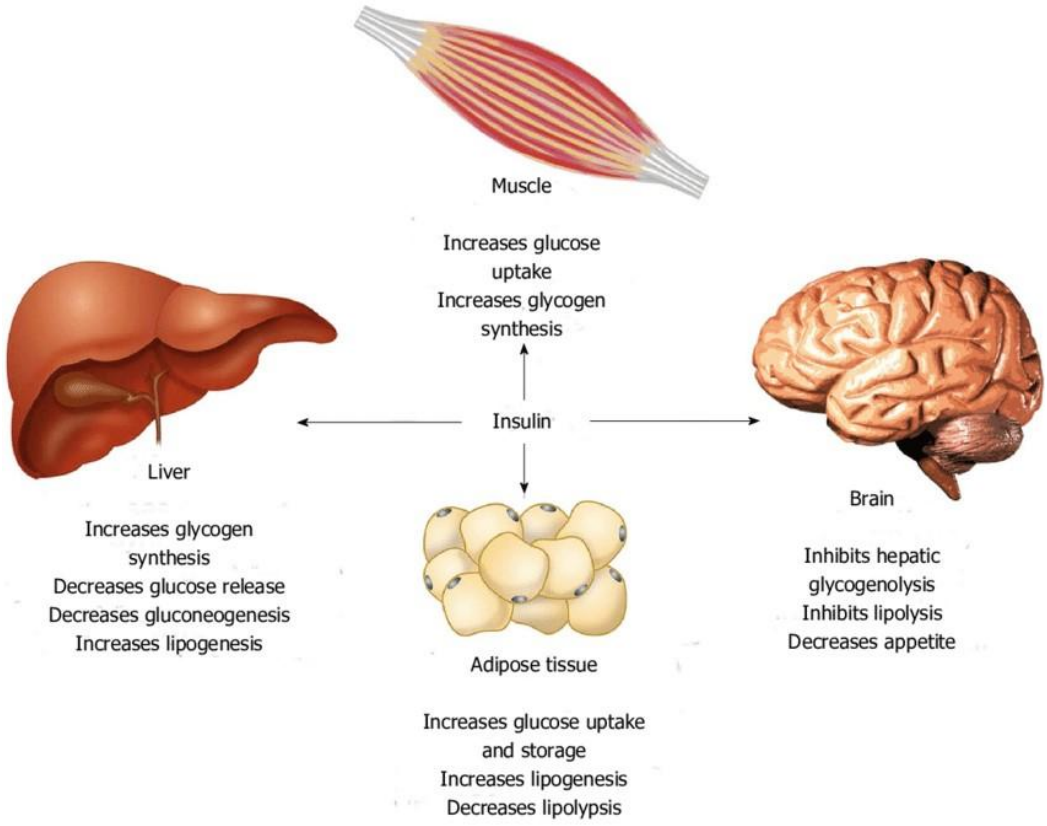


Figure 1.4. Insulin actions on main insulin-sensitive tissues. Adapted without permission (Bunner et al. 2014).

1.2 Types of Diabetes

Diabetes has two main types of diabetes vis-à-vis type 1 diabetes (T1D) and type 2 diabetes (T2D). A proposed class of diabetes is known as type 3 diabetes (T3D) or brain diabetes, and it has been associated with Alzheimer's disease (AD) (de la Monte and Wands 2008; Leszek et al. 2017; Shaw 2017; Suzanne 2014). Another common type of diabetes which occurs during pregnancy is gestational diabetes (GD). Diabetes insipidus is a rare type of diabetes that disrupts the synthesis, transportation and/or release of the anti-diuretic hormone, vasopressin (Perkins et al. 2006; Saborio et al. 2000). Other types of diabetes encompass specific types caused by various factors including

monogenic diabetes syndromes, diseases of the exocrine pancreas, endocrinopathies, and drugs/chemicals-induced diabetes (ADA 2015; Kerner and Brückel 2014).

1.2.1 Type 1 Diabetes

Type 1 diabetes accounts for 10% of the world's total diabetic cases (Maahs et al. 2010; Ozougwu et al. 2013). It is classified as an autoimmune disorder, where the immune system specifically targets and destroys the pancreatic β-cells, resulting in a complete lack of insulin and subsequent high blood glucose levels (ADA 2015; Maahs et al. 2010; Ozougwu et al. 2013). This condition was initially considered to be a metabolic disorder in children and adolescents, but it has since been found to affect adults as well. Polydipsia, polyphagia, and polyuria are regarded as the most common manifestation of its diagnosis (Atkinson 2012; Atkinson et al. 2014; Gregory et al. 2013). The autoimmune destruction of the pancreatic β – cells has been attributed to various factors, including genetics, immune responses, and environmental influences (Figure 1.5). The autoimmune destruction process is mediated by inflammatory cytokines such as macrophages, Tlymphocytes, B-lymphocytes, and β-cell autoantigens, dendritic cells (Burrack et al. 2017; Szablewski 2014; Xiang et al. 2023).

Multiple chromosomal loci have been pinpointed as contributing factors in the onset of T1D, with the human leukocyte antigen (HLA) locus being the most significantly predisposing factor (Lie et al. 1999; Noble and Valdes 2011). In addition to these loci, other locus implicated include the phosphatase non-receptor type 22 (PTPN22) loci, insulin, and cytotoxic T-lymphocyte antigen 4 (CTLA4) (Jahromi and Eisenbarth 2007; Kim and Polychronakos 2005; Pirot et al. 2008). These loci are located within the MHC-HLA class II region (Cucca et al. 2001; Hakonarson et al. 2007).

Additionally, the predisposing roles of MHC haplotypes and non-HLA loci have also been reported (Atkinson and Eisenbarth 2001; Barrett et al. 2009; Fernando et al. 2008). These genes provoke

an intensified inflammatory and immune response, which exacerbate the risk of autoimmunity and impair the β-cell function (Åkerblom et al. 2002; Pirot et al. 2008). Several environmental factors contribute to the development of T1D, such as infant and adolescent diets, lack of vitamin D, pre- and post-birth risks, climate, vaccinations, viral infections, toxins, and stress (Butalia et al. 2016; Chiacchiaretta et al. 2024; Giwa et al. 2020; Knip et al. 2010; Zorena et al. 2022). These environmental factors may modify a person's genetic predisposition, leading to the activation of cytokines that contribute to the autoimmune destruction of pancreatic β-cells (Houeiss et al. 2022; Stankov et al. 2013).

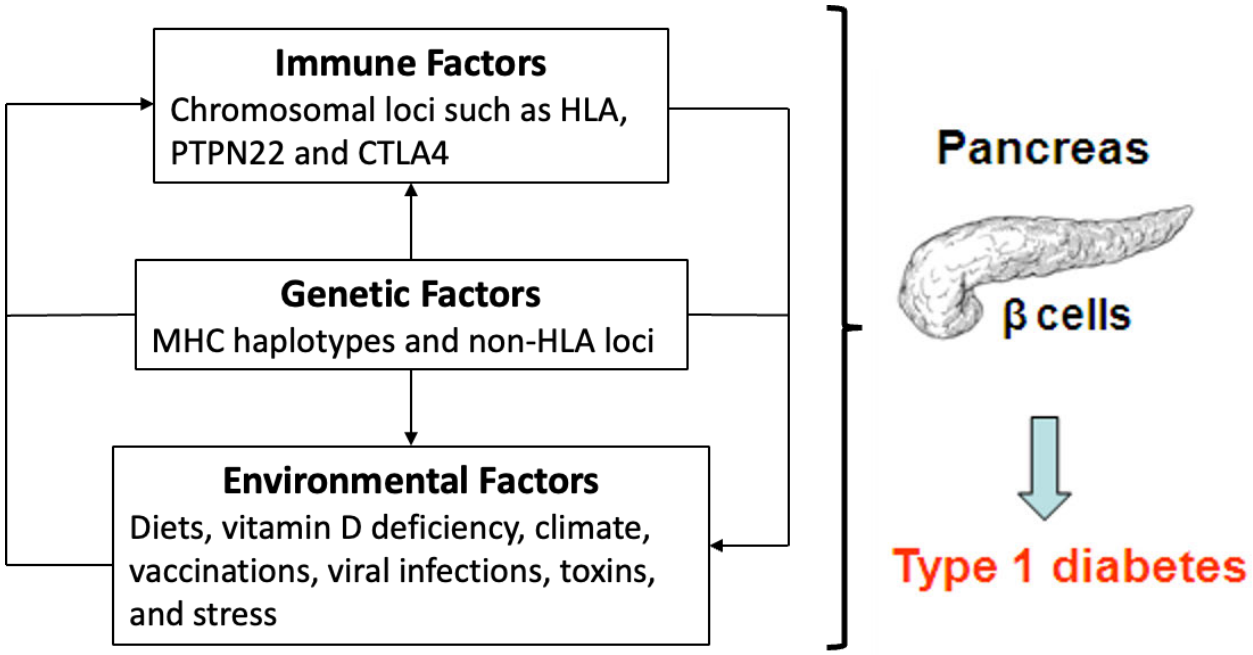


Figure 1.5. Factors contributing to autoimmune destruction of the pancreatic β – cells in the pathogenesis of Type-1 Diabetes.

1.2.2 Type 2 Diabetes

Type 2 diabetes is the most predominant of all diabetes types as it contributes to over 90% of morbidity and mortality related to diabetes (Erukainure et al. 2019; IDF 2021). It is a complex metabolic disorder arising from pancreatic β-cell dysfunction and insulin resistance which causes

inability of cells to utilize secreted insulin, thereby leading to hyperglycemia (Erukainure et al. 2019).

Both environment and genetic factors play interactive roles in the onset and progression of T2D (Murea et al. 2012). They include stress, overweight, energy-dense diets, physical inactivity, and sedentary lifestyle for environmental factors, while pancreatic β-cell dysfunction and insulin resistance for genetic factors (Figure 1.6) (Geng and Huang 2020; Kaul and Ali 2016; Murea et al. 2012).

Physical inactivity and overweight suppress the sensitivity of insulin, which in combination with a person's genetic predisposition to insulin resistance, causes the pancreatic β-cells to increase insulin production to compensate for the impaired insulin response (Defronzo 1997; DeFronzo 2009). This assists in preserving normal glucose tolerance levels. However, if prolonged, this compensation can lead to decreased pancreatic β-cell function with concomitant rise in fasting blood glucose level, resulting in insulin resistance and in the long run, diabetes (DeFronzo 2009).

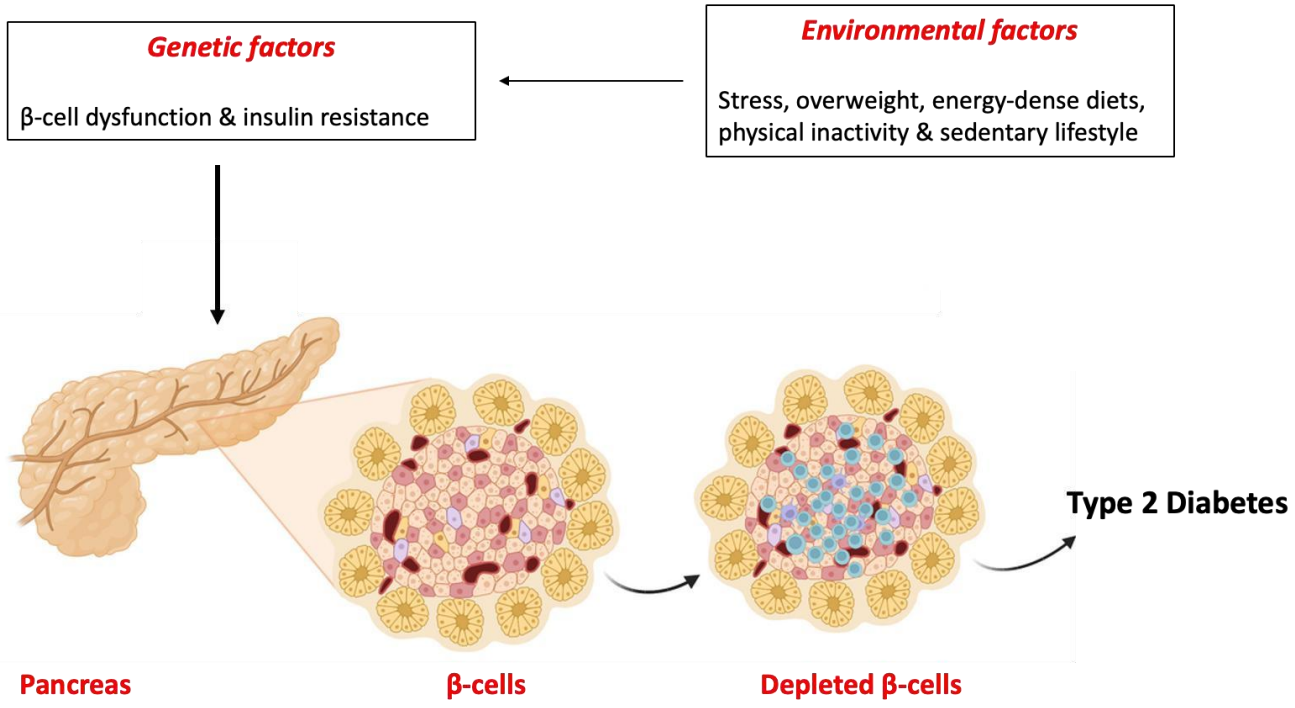


Figure 1.6. Interactions of environmental and genetic factors in the pathogenesis of Type-2 Diabetes.

1.2.3 Pathophysiology of Type 2 Diabetes

The ominous octet of β-cell, α-cell, adipocytes, gastrointestinal tract, kidney, liver, muscle and brain has been used in describing the pathophysiology of T2D (DeFronzo 2009; DeFronzo et al. 2015). As shown in Figure 1.7, they are involved in the eight major physiological abnormalities implicated in the onset and progression of T2D vis-à-vis insulin resistance (muscle and liver), impaired β-cell function, exacerbated glucagon secretion (α-cell), decreased incretin activity (gastrointestinal tract), adipocyte dysfunction, renal glucose reabsorption (kidney), and brain insulin resistance/neuro-dysfunction (DeFronzo 2018; Thrasher 2017).

Insulin resistance in the adipocytes increases lipolysis leading to the increased plasma free fatty acid (FFA) levels. This contributes to pancreatic β-cell failure by triggering insulin resistance in the liver and muscle (DeFronzo et al. 2015; Gustafson et al. 2015). This is further aggravated by resistance to the appetite-suppressive hormones, amylin, leptin and glucagon-like peptide 1 (GLP1) (DeFronzo 2009; DeFronzo et al. 2015) as well as low dopamine and serotonin levels in the brain (DeFronzo et al. 2015; Mooradian 1988), subsequently leading to hyperglycemia.

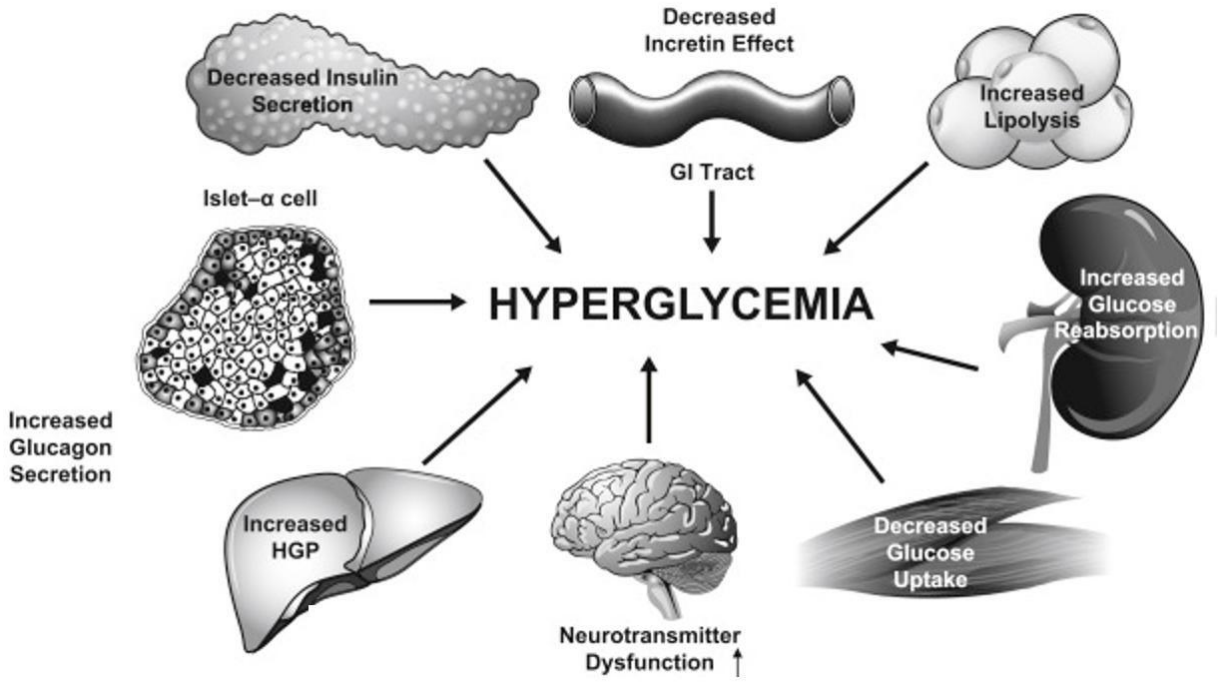


Figure 1.7. The ominous octet in the pathophysiology of Type-2 Diabetes. Adapted without permission (Thrasher 2017).

1.3 Complications of Type 2 Diabetes

As a multifactorial disease, T2D is associated with several complications, with hyperglycemia implicated in their pathogenesis. Chronic hyperglycemia increases the production of reactive oxygen species (ROS) and free radicals (Erukainure et al. 2017). The continuous production of ROS causes an imbalance between the body's endogenous antioxidant system and oxidant level, with the latter being favoured. This subsequently leads to oxidative stress. Oxidative stress has been reported as a major mechanism in the etiology and pathophysiology of T2D complications including such as hepatopathy, CVD, neuropathy, retinopathy, and nephropathy (Msomi et al. 2023; Xiao et al. 2023). Hyperglycemia-mediated inflammation also plays a major role in the etiology and pathophysiology of T2D complications via increased production of inflammatory cytokines (Catalfamo et al. 2013; Chang and Yang 2016).

1.3.1 Hyperglycemia and Oxidative Stress

Chromic hyperglycemia has been implicated in exacerbated production of ROS in T2D. Pancreatic β-cells are the most susceptible to oxidative stress, owing to low expression levels of glutathione peroxidase (GPx) and catalase (Pi et al. 2010). They also have a high expression of GLUT2, making them have high efficiency for glucose uptake (Kajimoto and Kaneto 2004), leading to excess glucose accumulation in T2D (glucotoxicity). In a transition-metal dependent reaction (Figure 1.8), the excess glucose undergoes enolization to generate enediol radical, which is further reduced to superoxide anion ($O_2^{\cdot -}$) and reactive ketoaldehydes radicals (Ahmed 2005; González et al. 2023). The generated $O_2^{\cdot -}$ is reduced by SOD to hydrogen peroxide (H_2O_2). Owing to its instability and suppression of the cells' glutathione peroxidase (GPx) and catalase activities, H_2O_2 from continuous glucose enolization in T2D, is further broken down to hydroxyl radicals ($OH^{\cdot}$) via the Fenton reaction (Meyerstein 2021). Reactive ketoaldehydes radicals reacts with the amino groups of tissue proteins to form ketoimine, which is further reduced to advanced glycation end products (AGEs), that subsequently binds to the AGE receptor (RAGE) (Ahmed 2005; González et al. 2023).

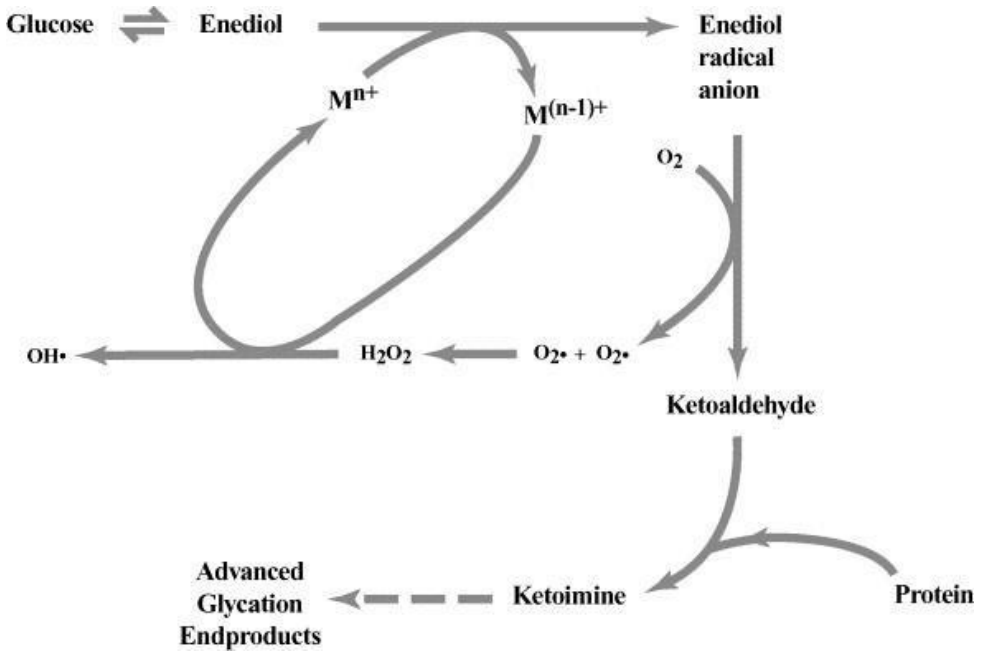


Figure 1.8. Enolization of glucose in glucotoxicity. Adapted without permission (Ahmed 2005).

Increased production of electron donors in both anaerobic and aerobic oxidation of glucose has also been implicated in glucotoxicity in T2D via generation of $O_2^{\cdot-}$. In hyperglycemic state, increased anaerobic oxidation of glucose, also known as glycolysis, leads to continuous production of lactate from pyruvate. This alters the balance of the cytosolic NADH/NAD+ ratio, impairing the oxidation of NADH to NAD+ (Chikezie et al. 2015). Thereby increasing cellular levels of NADH, an electron donor.

In aerobic oxidation of glucose, pyruvate (not converted to lactate) from glycolysis is channeled to the tricarboxylic acid (TCA) cycle where it generates NADH and FADH2. These electron donors are channeled to the electron transport chain (ETC) to generate energy, in the form of ATP (Brownlee 2001). In hyperglycemic state, enhanced aerobic oxidation causes increased production of these electron donors and setups a high mitochondrial membrane potential. The high membrane potential impairs electron transport at complex II of the ETC, leading to the reduction of oxygen to (O_2) to $O_2^{\cdot-}$ (Brownlee 2001; Du et al. 2001).

Other pathways such as the polyol, hexosamine, and protein kinase C (PKC) pathways have been implicated in hyperglycemia-induced oxidative stress in T2D (Erukainure and Chukwuma 2024; Erukainure et al. 2024).

In the polyol pathway, excess glucose arising from hyperglycemia undergoes reduction to sorbitol in a reaction catalyzed by aldose reductase. Sorbitol is further oxidized to fructose by sorbitol dehydrogenase (Erukainure and Chukwuma 2024). Fructose in turn is channelled to the methylglyoxal pathway to produce AGEs. Aldose reductase also suppresses the oxidation of NADH to NAD+, leading to increased production of the electron donor and suppressed cellular levels of glutathione (Brownlee 2005). Thereby, contributing to oxidative stress.

In the hexosamine pathway, fructose-6-phosphate from the glycolytic pathway is converted by glutamine: fructose-6 phosphate amido transferase (GFAT) to glucosamine-6-phosphate and subsequently, uridine diphosphate (UDP) *N*-acetyl glucosamine (Aronson 2008; Brownlee 2005). UDP *N*-acetyl glucosamines have been implicated in O-glycosylation of proteins by the addition of N-acetylglucosamine (GlcNAc) group to their serine and threonine residues. O-glycosylation alters the activities of enzymes and other proteins leading to formation of carotid artery plaques, cardiomyocyte dysfunction, and abnormal glomerular cell gene expression (Aronson 2008; Brownlee 2001; Brownlee 2005; Goldin et al. 2006).

In the PKC pathway, glyceraldehyde-3-phosphate and dihydroxy-acetone phosphate are channelled from the glycolytic pathway to form diacylglycerol under hyperglycemic state (Aronson 2008). Diacylglycerol is an important co-factor for the activation of PKC isoforms: α, β, and γ (Brownlee 2001; Brownlee 2005). These activations in turn activate NADPH oxidase which is involved in the generation of ROS (Inoguchi et al. 2000).

Together, these pathways (Figure 1.10) and glucose enolization portray potential unifying mechanism underlying hyperglycemia-mediated oxidative stress which has been implicated in the pathophysiology of T2D and complications.

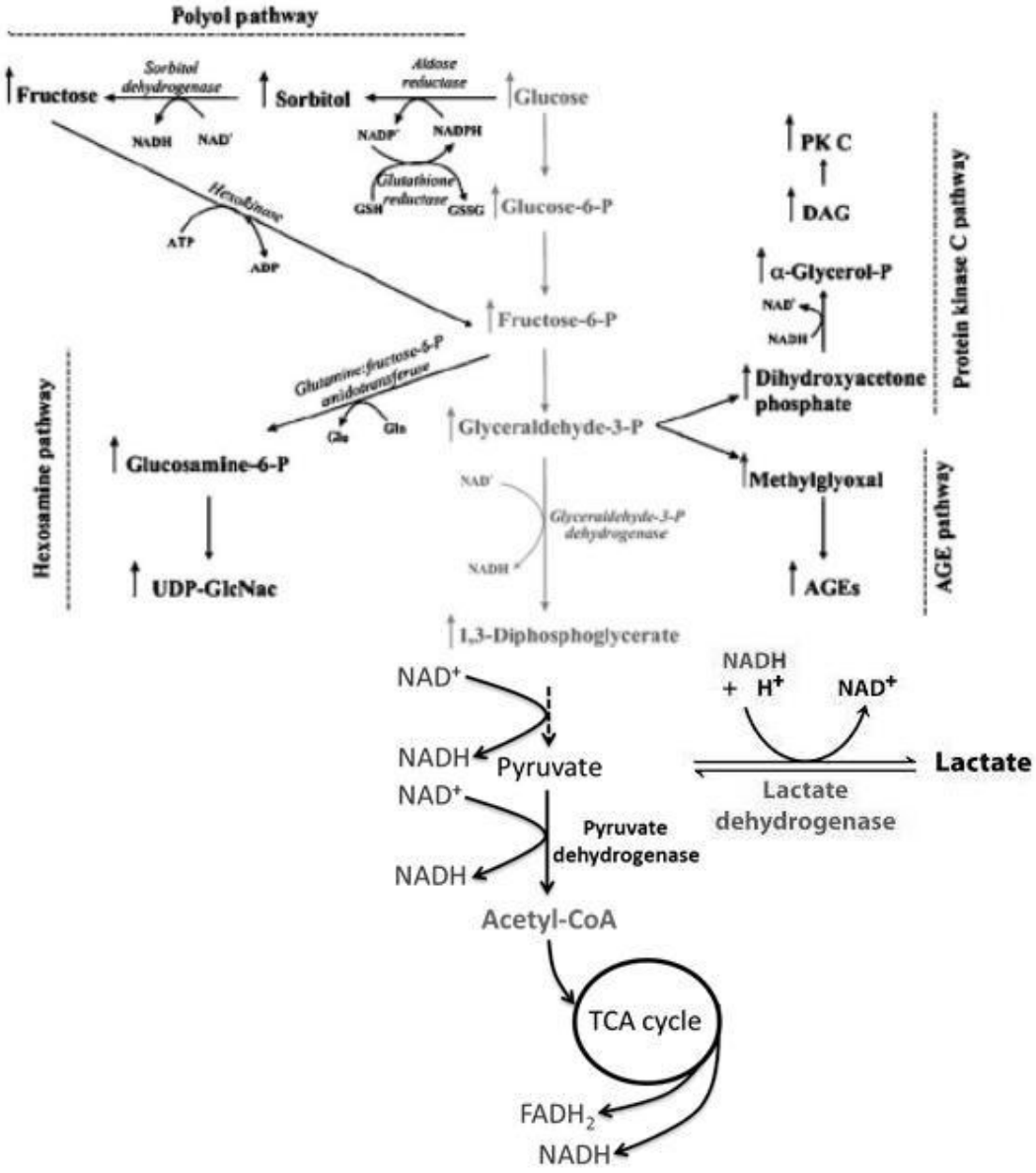


Figure 1.9. Pathways implicated in hyperglycemia-mediated oxidative stress in Type-2 Diabates. Adapted without permission (Luo et al. 2016; Rolo and Palmeira 2006).

1.4 Diabetic Hepatopathy

There have been increasing concerns on the increasing prevalence of hepatopathy (liver disease) in people living with T2D (Islam et al. 2020; Mobasheri et al. 2023). Diabetic hepatopathy is a microvascular complication of T2D and a major contributor to T2D mortalities (Mallet et al. 2022; Tolman et al. 2007). Diabetic hepatopathy is a spectrum of liver diseases including nonalcoholic fatty liver disease (NAFLD), hepatocellular carcinoma, abnormal liver enzymes, cirrhosis, and acute liver failure (Islam et al. 2020; Mobasheri et al. 2023; Tolman et al. 2007). Cirrhosis accounts for 4.4% of diabetes-related deaths, making it the fourth leading contributor to mortality in T2D according to a population-based Verona diabetes study (De Marco et al. 1999; Tolman et al. 2007). NAFLD has been reported in 37–55% of T2D patients, and increases the risk of hepatic fibrosis in these individuals (Barb et al. 2021; Mobasheri et al. 2023).

1.5 The Liver and Glucose Homeostasis

The liver is the main organ responsible for the body's metabolism including glucose homeostasis. After food consumption, glucose is absorbed into the liver and stored as glycogen. During fasting, stored glycogen is broken down to glucose via glycogenolysis, while glucose is also generated via gluconeogenesis (Adeva-Andany et al. 2016). Glucose arising from the □-amylases and □glucosidase-mediated hydrolysis of dietary carbohydrate, is transported into the hepatocytes via glucose transporter-2 (GLUT2). The transported glucose is phosphorylated by glucokinase to glucose-6-phosphate (G6P), which is an allosteric activator of glycogen synthase in the glycogenic pathway. Glucokinase expression is tightly upregulated by insulin via the sterol regulatory element binding proteins (SREBPs) (Mobasheri et al. 2023). Glycogen synthase is also activated by insulin via the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway. The pathway inhibits the activity

of glycogen synthase kinase 3 (GSK3), thus driving glycogenesis while arresting glycogenolysis (Iynedjian et al. 1995). In fasting state, glycogen phosphorylase and phosphoenolpyruvate carboxykinase (PEPCK) drives gluconeogenesis.

Glucose 6-phosphate can also be channelled to the glycolytic pathway or hexosamine pathway via conversion to fructose 6-phosphate to yield acetyl-CoA and uridine diphosphate-Nacetylglucosamine, respectively. Acetyl-CoA may further be channelled to the tricarboxylic acid (TCA) cycle in the mitochondria for energy production or into the cytosol for lipogenesis (AdevaAndany et al. 2016). Insulin plays a regulatory role on lipogenesis, via the oxysterol-sensing transcription factor liver X receptor (LXR) which controls the transcription of SREBPs (Han et al. 2016). SREBPs are the major regulators of lipid metabolism in mammals as they stimulate the transcription of genes involved in the expression of cholesterol biosynthesis, 3-hydroxy-3methylglutaryl-coenzyme A reductase (HMGCR), hydroxy-3-methylglutaryl-coenzyme A synthase 1 (HMGCS1), low density lipoprotein receptor (LDLR), and farnesyl diphosphate synthase (FDPS) (Han et al. 2016; Mobasheri et al. 2023). High glucose concentration has also been shown to upregulate SREBPs independently of insulin (Im et al. 2005; Mobasheri et al. 2023). This is summarized in Figure 1.11.

Furthermore, glucose 6-phosphate can be channelled to the production of ribose 5-phosphate and nicotinamide adenine dinucleotide phosphate (NADPH) via the pentose phosphate pathway. The former is utilized in the synthesis of RNA or DNA, while the latter is required as a co-factor for lipogenesis and maintenance of cellular reduced glutathione (GSH) (Adeva-Andany et al. 2016).

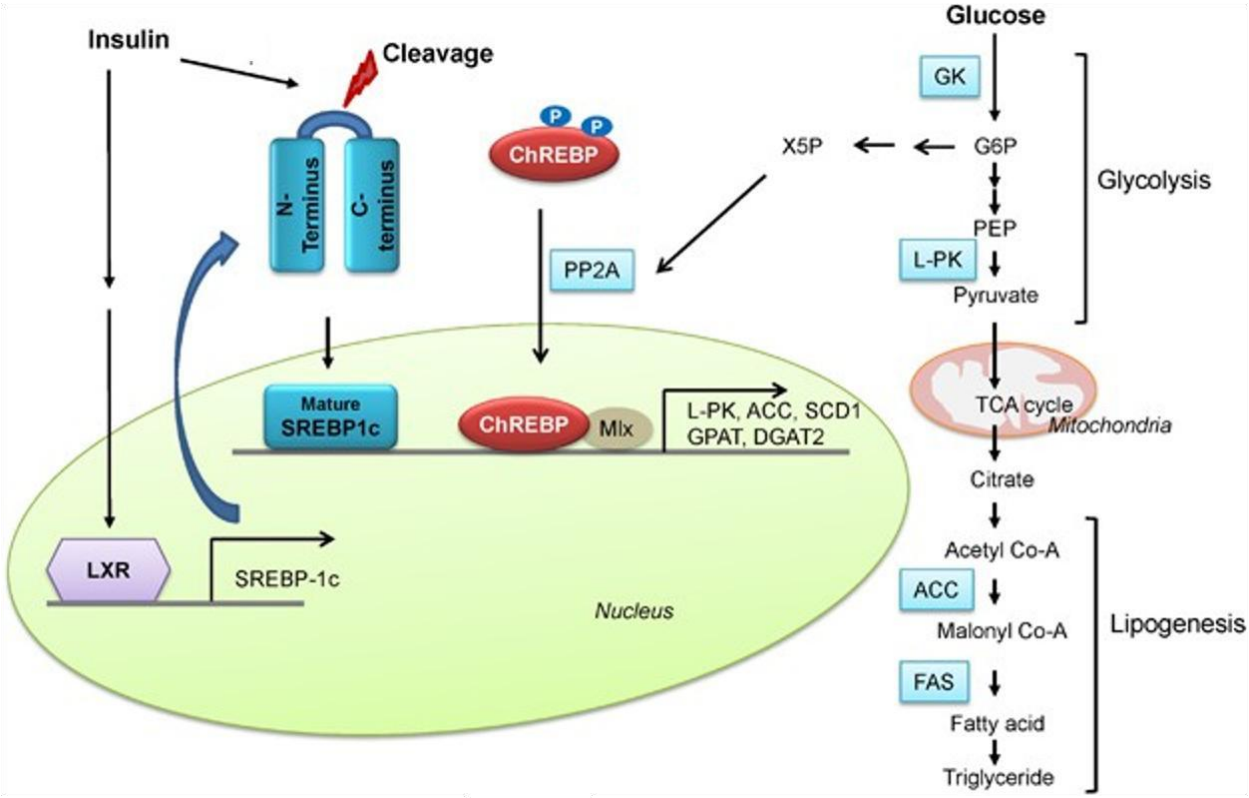


Figure 1.10. Regulation of hepatic glycolysis. Adapted without permission (Han et al. 2016).

1.6 Pathophysiology of Diabetic Hepatopathy

The pathophysiology of diabetic hepatopathy has been linked to hyperglycemia, dyslipidaemia, insulin resistance and hyperinsulinemia (Mobasheri et al. 2023). High glucose uptake in the liver in hyperglycemic state increases the hepatic concentration of G6P. High concentration of circulating insulin stimulates the production of glycogen from G6P. This causes a high cellular level of glycogen which gets stored in the hepatic walls, leading to the leakage of liver enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) into the blood (Giordano et al. 2014). High serum/plasma levels of these enzymes in diabetes are considered as markers of insulin resistance (Bulum et al. 2011).

Insulin resistance induces adipocyte lipolysis leading to increased blood levels of fatty acids, which is transported to the hepatocytes. Insulin resistance further stimulate de novo lipogenesis in the hepatocytes, while concomitantly suppressing hepatic fatty acid oxidation and secretion of very low-density lipoprotein (VLDL) (Mobasheri et al. 2023; Utzschneider and Kahn 2006). Thereby causing triglycerides accumulation in the liver, and leading to the development of steatosis and NAFLD (Utzschneider and Kahn 2006).

Increased hepatic levels of glucose and lipids exacerbate the production of ROS and other free radicals, leading to oxidative stress. Excessive hepatic glucose can be channelled to other carbohydrate metabolic pathways such the polyol, hexosamine and PKC pathways to generate toxic metabolites such as AGEs, UDP *N*-acetyl glucosamines and other percussors for the generation of free radicals (Garcia-Compean et al. 2009). Depletion of NADPH via the polyol pathway will also lead to an imbalance in the reduction of glutathione, further increasing the cells susceptibility to oxidative stress.

1.7 Management of Diabetic Hepatopathy

Modification of lifestyle and diets, especially focused on weight loss, have been shown to suppress leakage of liver enzymes and hepatic lipid content in individual with T2D (Suzuki et al. 2005). However, these modifications are often combined with pharmacological interventions focused on reducing hyperglycemia and improving insulin sensitivity. Biguanides, sulfonylurea, dipeptidyl peptidase (DPP) IV inhibitors and thiazolidinediones, are the major drug classes utilized as pharmacological intervention in the management of diabetic hepatopathy (Sharabi et al. 2015; Utzschneider and Kahn 2006).

1.7.1. Biguanides

This is a class of therapeutic drugs that modulates hyperglycemia by suppressing hepatic glucose output, with concomitant increase in insulin-mediated muscle glucose uptake and utilization (Campbell 2007; Tripathi and Srivastava 2006). Metformin is the most common drug in this class. It was originally isolated in the 1920s from the *Galega officinalis* plant and ranks among the most prescribed anti-hyperglycemic drug globally (Martin and Marais 2012). Metformin exerts its antidiabetic effect primarily by arresting gluconeogenesis, leading to reduced hepatic glucose production. It also impairs ATP production by inhibiting complex I activity in the mitochondria (El-Mir et al. 2000; Owen et al. 2000). This leads to increased AMP levels which inhibits adenylyl cyclase catalyzed cAMP production, there by suppressing the hepatic glucose production and inactivating glucagon signaling (Miller et al. 2013). Subsequently leading to reduced glucose output. Metformin has also been shown to suppress gluconeogenesis from glycerol and lactate by inhibiting glycerol 3-phosphate dehydrogenase activity in the mitochondrial, which causes a shift in the $NADH/NAD^+$ ratio (Madiraju et al. 2014).

However, metformin is associated with side effects such as nausea, diarrhea, lactic acidosis, abdominal spasm, and metallic aftertaste (Bösenberg and van Zyl 2008).

1.7.2. Sulfonylurea

This is a class of therapeutic drugs that reduces blood glucose level by stimulating insulin secretion from pancreatic β-cell. They cause closure of ATP-sensitive K^+ channels by binding directly to the β-cells through the transmembrane sulphonylurea receptor (SUR-1) (Bösenberg and van Zyl 2008;

Tripathi and Srivastava 2006). This causes a reduction in potassium efflux which depolarizes the membrane and opens the voltage-dependent $Ca2^{+}$-channels (Bösenberg and van Zyl 2008). This leads to calcium influx that stimulates the release of insulin from pancreatic secretory granules (Sharabi et al. 2015). Members of this drug class include glibenclamide, glipizide, glimepiride, and gliclazide. However, the use of this drug class has been associated with hypoglycemia and modest weight gain (Chiniwala and Jabbour 2011; Sharabi et al. 2015).

1.7.3. Dipeptidyl peptidase (DPP) IV inhibitors

This is a class of antidiabetic drugs that reduces blood glucose level by inhibiting the activities of dipeptidyl peptidase IV (DPP-4), an antagonist of incretin hormones such as the glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) (Osadebe et al. 2015; Pratley and Salsali 2007). The inhibition of DDP-4 activity increases glucose-dependent insulin secretion, while concomitantly suppressing the release of glucagon and gastric emptying, thereby leading to reduced blood glucose level (Holst 2007). Members of this drug class include linagliptin, vidagliptin, sitagliptin, alogliptin, and saxagliptin. The use of this drug class has been associated with acute pancreatitis, pharyngitis, and headache (Erukrainuire et al. 2019).

1.7.4. Thiazolidinediones

This is a class of antidiabetic drugs that reduces blood glucose level by improving insulin sensitivity. They exert their effect by binding to the peroxisome proliferator-activated receptor gamma (PPAR-γ) receptor (Bösenberg and van Zyl 2008). The activation of PPAR-γ, a transcription factor stimulates insulin-dependent suppression of hepatic glucose output (Tripathi and Srivastava 2006). It also suppresses hepatic lipogenesis by reducing hepatic fatty acids uptake

and enhancing adiponectin levels which stimulates fatty acid oxidation (Mayerson et al. 2002; Yu et al. 2002).

Members of this drug class include troglitazone, rosiglitazone, and pioglitazone. However, the use of this drug class has been associated with hypoglycemia, weight gain, and oedema. Increased risks of bladder cancer and non-fatal heart attack have been implicated with the use of pioglitazone and rosiglitazone, respectively (Bösenberg and van Zyl 2008).

1.8 Natural Products in the Management of Diabetic Hepatopathy

Over the years there have been increasing interests in the use of natural products of plant origins in the management of T2D and its complications including diabetic. This paradigm shift from conventional synthesized medications has been attributed to availability of these natural products at affordable costs, coupled with little or no side effects associated with their use. Among these natural products are polysaccharides.

1.9 Polysaccharides

Polysaccharides are carbohydrate molecules made up of long chains of monosaccharide units polymerically linked together by glycosidic bonds (Wu et al. 2016). Their general formular are presented as $(C_6H_{10}O_5)_n$, where n ranges between 40 – 3000. Polysaccharides function as energy storage units, structural supports, and signaling molecules within the cell walls of plants, fungi, bacteria, and algae (Fernandes and Coimbra 2023). They occur in animal cells as components of the extracellular matrix, and also comprise their external skeletons (Lovegrove et al. 2017). In foods, they serve as energy source and may influence the sensory qualities of food, particularly its texture and mouthfeel (Yang et al. 2020).

The structure of polysaccharides can be linear or highly branched. Their structures have been reported to play important roles in their biological and medicinal functions such as hepatoprotection, immunomodulation, antioxidant, radioprotection, and antimicrobial activities (Fernandes and Coimbra 2023; Wu et al. 2016). The antidiabetic effect of polysaccharides has been demonstrated in several studies. They exhibit this effect via several mechanisms including reduction of fasting blood glucose, enhancement of insulin secretion, regeneration of β cell, attenuation of oxidative stress and inflammation, and inhibition of protein-tyrosine phosphatase 1B (PTP1B) as summarized in Table 1.1.

Table 1.1: Antidiabetic properties of polysaccharides.

Antidiabetic activity	Polysaccharide name	Monosaccharide composition	References
Hypoglycemic and hypolipidemic effects	*Acacia tortilis* polysaccharide	D-galactose, d-glucose, lrhamnose, and d-glucuronic acid	(Kumar Bhateja and Singh 2014)
	Lachnum calyculiforme (LEP-1)	(1 →3)-β-glucan	(Ye et al. 2011)
	Mactra veneriformis (CPPM)	Glucose, sulfonic and uronic acids	(Wang et al. 2011)
	Schisandra chinensis (Turcz.) *Baill* (ESCPs)	D-rhamnose, l-arabinose, dxylose, d-glucose, d-galactose, and d-mannose	(Zhao et al. 2013)
	Sulphated polysaccharide	Fucose, sulphate, uronic acid, galactose, mannose, glucose and arabinose	(Wang et al. 2013b)
	Inula *britannica* flower polysaccharide (IBP)	Mannose, glucuronic acid, rhamnose, galacturonic acid, glucose, galactose and arabinose	(Hong et al. 2012)
	Pumpkin polysaccharide (PCE-F)	Glucose, galactose, arabinose and rhamnose	(Jin et al. 2013)
	Talinum triangulare polysaccharides (TTPs)	Rhamnose, arabinose, mannose and galactose	(Xu et al. 2015b)
β cell regeneration/ Inhibition of pancreatic β-cell apoptosis	Mulberry leaf polysaccharides (MLPII)	D-arabinose, d-xylose, dglucose, d-rhamnose, and dmannose	(Zhang et al. 2014)
	Ganoderma atrum polysaccharide (PSG-1)	Glucose, mannose, galactose and galcturonic acid	(Zhu et al. 2016; Zhu et al. 2013b)

	Ganoderma lucidum protein-bound polysaccharide (Gl-PS)	Glucose, galactose, arabinose and mannose	(Li et al. 2011; Zheng et al. 2012)
	Fucosylated chondroitin sulphate	Glucuronic acid, galactosamine and fucose	(Hu et al. 2014a)
	Lycium barbarum acidic polysacharride (LBP-s-1)	Rhamnose, arabinose, xylose, mannose, glucose, galactose, and galacturonic acid	(Zhu et al. 2013a)
Enhanced insulin secretion	*Ophiopogon japonicus* β-d-fructan (MDG-1)	Glucose	(Wang et al. 2012)
	Liriope spicata var. *prolifera* polysaccharides	-(1 -⟶ 2)-fructosyl residues	(Liu et al. 2013c; Xiao et al. 2014)
	Grifola frondosa polysaccharide (GFP)	Rhamnose, arabinose, xylose, mannose, glucose and galactose	(Ma et al. 2014)
	Lycium barbarum fruit polysaccharides (LBP-4a)	Galactose, glucose, rhamnose, arabinose, mannose and xylose	(Zhao et al. 2014)
	Ganoderma atrum polysaccharide (PSG-1)	1,3- and 1,6-Glcp branched at the O-3 and O-6 positions	(Liu et al. 2012; Zhu et al. 2014)
	Sea cucumber fucosylated chondroitin sulphate derived (SC-CHS)	Chondroitin sulphate E backbone and sulphated fucose	(Hu et al. 2013b; Hu et al. 2013c)
	Enteromorpha prolifera polysaccharides (PEPs)	Rhamnose, glucuronic acid, arabinose, fucose, xylose and glucose	(Lin et al. 2015)

	Tea polysaccharide (TPS)	L-arabinose, d-ribose, d-xylose, dglucose and d-galactose	(Li et al. 2015)
	Acaudina molpadioides fucosylated chondroitin sulphate (Am-CHS)	Glucuronic acid, galactosamine and fucose	(Hu et al. 2014b)
	Ramulus mori polysaccharide (RMP)	Glucose, galactose, mannose, rhamnose and arabinose	(Xu et al. 2015a)
Inhibition of protein-tyrosine phosphatase 1B (PTP1B)	FYGL-n	D-arabinose, d-galactose, lrhamnose, and d-glucose	(Pan et al. 2015)
	Grifola frondosa protein-bound polysaccharides (F2, F3)	**F2**: glucose, mannose, galactose, xylose, arabinose, rhamnose and ribose **F3**: ribose, arabinose and xylose	(Xiao et al. 2015)
Attenuation of oxidative stress	*Catathelasma ventricosum* polysaccharide (CVPS)	Fucose, mannose and galactose	(Liu et al. 2013a; Liu et al. 2013b)
	Ophiopogon japonica polysaccharide (OJP1)	Glucose, galactose, and arabinose	(Chen et al. 2011)
	WGPA and WGPN	**WGPA**: galactose, glucose, arabinose and galacturonic acid **WGPN**: galactose, glucose and arabinose	(Sun et al. 2014)
	Pleurotus florida polysaccharide (PFP)	glucose, galactose, mannose, arabinose and xyliyol	(Ganeshpurkar et al. 2014)
Attenuation of inflammation	*Angelica sinensis* polysaccharide (ASP)	Arabinose, glucose and galactose	(Hotamisligil 2000)

	Pseudostellaria heterophylla polysaccharide (PF40)	Glucose, galactose, galacturonic acid, arabinose, and rhamnose	(Hu et al. 2013a)
	Pleurotus sajor-caju polysaccharide (GE)	α-glucan and β-glucan	(Kanagasabapathy et al. 2012)

1.10 Polysaccharides and Diabetic Hepatopathy

The use of polysaccharides in the management of diabetic hepatopathy has been well documented. This has been attributed to their ability to improve liver function, modulate glucose and lipid metabolism, and mitigate oxidative stress (Bai et al. 2023; Li et al. 2020; Liu et al. 2020; Wang et al. 2018). Recent studies on the therapeutic mechanisms of polysaccharides on diabetic hepatopathy is summarized in Table 1.2.

Table 1.2: Recent studies on the therapeutic mechanisms of polysaccharides on diabetic hepatopathy

Polysaccharides	Monosaccharide composition	Mechanism of action	Diabetic models	References
Rehmannia glutinosa polysaccharides (RGP)	Rhamnose, arabinose, mannose, glucose, and galactose	Increased hepatic gluconeogenesis and glycogen content, with concomitant reversal of increased mRNA expression of PEPCK	STZ-induced diabetic mice	(Zhou et al. 2015a)
Misgurnus anguillicaudatus polysaccharide (MAP)	Galactose, fucose, and mannose			(Zhou et al. 2015b)
Ganoderma lucidum (GLP)	Glucose and mannose	Suppressed the hepatic expression of PEPCK, glycogen phosphorylase, G6Pase and FBPase mRNAs	STZ/HFD-induced diabetic mice	(Xiao et al. 2012)
		Reduced serum levels of hepatic functional indicators (ALT and AST); improved hepatic morphology, and mitigated hepatic inflammation by modulating the Nrf2/HO-1 signaling pathway	HFD-fed C57BL/KsJ*db/db* male mice	(Li et al. 2020)
Lycium barbarum polysaccharide (LBP-s-1)	Rhamnose, arabinose, xylose, mannose, glucose, galactose, galacturonic acid	Enhance hepatic glycolysis by increasing the activities of hepatic hexokinase and pyruvate kinase	STZ-induced diabetic mice	(Zhu et al. 2013a)
Mulberry leaf polysaccharide (MLPII)	D-arabinose, d-xylose, dglucose, d-rhamnose, and d-mannose	Improved hepatic glycolysis by activating hepatic GCK. Suppressed hepatic PTP1B	STZ-induced diabetic rats	(Ren et al. 2015; Xu et al. 2015a; Zhang et al. 2014)

		mRNA and enhanced IRS2 mRNA		
Opuntia dillenii polysaccharide (ODP-Ia)	Glucose, galactose, rhamnose and arabinose, with an arabinuronic acid	Increased hepatic glycogen levels, HDL-C levels, with concomitant attenuation of hepatic oxidative stress by increasing SOD and GSH-Px activities	STZ-induced diabetic mice	(Zhao et al. 2011)
Red kidney bean polysaccharide (RKPH)	Amylose and amylopectin	Reduced serum levels of hepatic functional indicators (ALT, ALB, AST and TP) and improved hepatic lipid metabolism	STZ- and HFDinduced T2D mice	(Bai et al. 2023; Singh 2024)
Small black soybean polysaccharide (SBPH)	Glucose, rhamnose, galacturonic acid, mannose, and galactose			(Bai et al. 2023; Huang et al. 2023)
Astragalus polysaccharides (APS)	Rhamnose, galacturonic acid, glucose, galactose, and arabinose	Upregulated hepatic miR-203a-3p, with concomitant suppression of hepatic GRP78, CHOP, pJNK1 and caspase-12 protein expression levels and improved morphology	T2DM GK rats	(Wang 2017; Wei et al. 2018)

Dendrobium officinale polysaccharide (DOP)	D-glucose and d-mannose	Improved hepatic glucose metabolism by modulating liver glycogen structure, inhibiting hepatic glycogen degradation and hepatic gluconeogenesis, restoring hepatic histology, and suppressing the expressions of	HFD/STZ-induced T2D mice	(Liu et al. 2020)
		hepatic GCGR, AC, PKA-C, p-PKA, p-GS, GBE and GP.		
Pleurotus djamor acidic-, alkalic and enzymatic extractable mycelium zinc polysaccharides (Ac-MZPS, AlMZPS and EnMZPS)		Mitigated oxidative stress in hepatic tissue, while restoring hepatic morphology and reducing serum levels of hepatic functional indicators (ALT, ALB, and AST).	STZ-induced diabetic mice	(Zhang et al. 2015b)
Grifola frondosa polysaccharide (GFP-N)	L-arabinose, d-mannose and d-glucose	Improved hepatic insulin resistance via modulation of JNK and IRS1/PI3K signaling	HSFD-induced diabetic mice	(Chen et al. 2019b)
Suillellus luridus polysaccharide	Mannose, arabinose, xylose and galactose	Mitigated oxidative stress in hepatic tissue, while restoring hepatic morphology	STZ-induced diabetic mice	(Zhang et al. 2018)

Liriope spicata var. prolifera tuberous root polysaccharides (LSP1, LSP2 and TLSP)	β-(1-2)-fructosyl residues	Improve hepatic glucose metabolism by suppressing gluconeogenesis and increasing hepatic glycolysis and hepatic glycogen content. It also restored hepatic morphology	KKAY mice	(Liu et al. 2013c)
Schisandra chinensis (Turcz.) *Baill* polysaccharides (ESCPs)	L-rhamnose, larabinose, d-xylose, dglucose, d-galactose, and d-mannose	Increased hepatic glycogen content, and improved lipid metabolism	Alloxan-induced diabetic mice	(Zhao et al. 2013)
Inula britannica flower polysaccharide (IBP)	Mannose, glucuronic acid, rhamnose, galacturonic acid, glucose, galactose and arabinose	Increased hepatic glycogen content, and improved lipid metabolism	Alloxan-induced diabetic mice	(Hong et al. 2012)
Salvia miltiorrhiza Bunge polysaccharide (SMPW1)	Glucose, arabinose, rhamnose, and galactose	Mitigated hepatic oxidative stress and restored hepatic morphology	High fat and high glucose diet (HGF)-induced insulin resistant rats	(Ke et al. ; Zhang et al. 2012)
Catathelasma ventricosum polysaccharide (CVPS)	Fucose, mannose and galactose	Mitigated hepatic oxidative stress, while increasing vitamin E content	STZ-induced diabetic mice	(Liu et al. 2013a; Liu et al. 2013b)
Catathelasma ventricosum selenium polysaccharide (SPC-2)	Glucose	Mitigated hepatic oxidative stress and restored hepatic morphology	STZ-induced diabetic mice	(Liu et al. 2013a)

Ophiopogon japonica (OJP1)	Glucose, galactose, and arabinose	Mitigated oxidative stress and modulated growth factor-β1 and connective tissue growth factor in hepatic tissues	STZ-induced diabetic rats	(Chen et al. 2011)
Anoectochilus roxburghii polysaccharides (ARP)	L-rhamnose, larabinose, d-xylose, dmannose, d-glucose, and dgalactose	Attenuated hepatic pathologic lesions, while reducing serum levels of hepatic functional indicators (ALT and AST).	STZ-induced diabetic mice	(Zhang et al. 2015a)
Artemisia sphaerocephala Krasch (seed) polysaccharides (ASKP)	D-mannose, d-glucuronic acid, d-galacturonic acid, d-glucose, dxylose, d-galactose, larabinose	Suppressed hepatic lipid level, while mitigating hepatic oxidative stress and steatosis	High-fructose induced hyperglycosemia mice	(Ren et al. 2014)
Liriope spicata var. *prolifera* polysaccharides (TLSP, LSP1, and LSP2)		Mitigated hepatic insulin resistance by enhancing InsR/IRS1/PI3K insulin signaling pathway in livers,	KKAy mice	Liu et al. 2013c; Xiao et al. 2014)
		and increasing hepatic GLUT4 expression		
Sea cucumber fucosylated chondroitin sulphate (SC-CHS)	Chondroitin sulphate E backbone and sulphated fucose	Improved hepatic glucose metabolism via the PI3K/PKB/GSK-3β pathway and increased glycogen synthase activity	Insulin-resistant C57BL/6J mice	(Hu et al. 2013b)

Enteromorpha prolifera polysaccharides (PEPs)	Rhamnose, glucuronic acid, arabinose, fucose, xylose and glucose	Exacerbated hepatic expressions of InsR and GCK mRNAs	High-sugar, highfat diet/STZinduced diabetic rats	(Lin et al. 2015)
Tea polysaccharide (TPS)	L-arabinose, d-ribose, dxylose, d-glucose and dgalactose	Mitigated hepatic oxidative stress and improved hepatic gluconeogenesis and insulin signaling via upregulation of PI3Kp85, p-Akt and GLUT4 expressions, while restoring hepatic morphology	STZ-induced diabetic mice	(Li et al. 2015)
Grifola frondosa protein-bound polysaccharides (F2, F3)	**F2**: glucose, mannose, galactose, xylose, arabinose, rhamnose and ribose **F3**: ribose, arabinose and xylose	Increased hepatic IRS-1 (ser307), IR, IRS-1, PI3K and AKT mRNA levels	HFD/STZ-induced diabetic rats	(Xiao et al. 2015)
Enterobacter cloacae Z0206 polysaccharides (EPS)	L-fucose, d-glucose, dgalactose, d-glucuronic acid and pyruvic acid	Increased hepatic expressions of ATGL, HST, CPT1-α and GK and reduced FAS and G6P gene expression	KKAY mice	(Huang et al. 2015; Wang et al. 2013a)

Low molecular weight fucoidan (LMWF)	Fucose and uronic acid	Mitigated hepatic oxidative stress and inflammation, and hepatic dysfunction; while modulating hepatic lipid metabolism and reversing abnormal hepatic SIRT1/AMPK/PGC1α signaling.	Obese diabetic db/db mice	(Zheng et al. 2018)
Gracilaria lemaneiformis polysaccharides (GLP)	Galactose, glucose, fucose and mannose	Improved hepatic lipid metabolism via upregulation of AMPKα, SREBP-2, HMG, LxRα and CYP7A1 genes expression; with concomitant restoration of hepatic morphology.	HFD-fed mice	(Huang et al. 2019)
		Improved hepatic index, reduced hepatic functional indicators (ALT and AST) and improved hepatic glucose metabolism	STZ-induced diabetic mice	(Wen et al. 2017)
		Mitigated hepatic oxidative stress and restored hepatic morphology	Alloxan-induced diabetic mice	(Liao et al. 2015)
Gracilaria lithophile polysaccharide		Mitigated hepatic oxidative stress	STZ-induced diabetic rats	(Lu et al. 2023)

Laminaria japonica Fucoidan (LJF)	Fucose, galactose, mannose, xylose, rhamnose, glucose, and glucuronic acid.	Reduced hepatic index, while increasing hepatic glycogen content and mitigating hepatic oxidative stress. It also restored hepatic morphology	HFD/STZ-induced diabetic mice	(Ponce and Stortz 2020; Zhang et al. 2022)
Sargassum horneri polysaccharides		Modulated hepatic lipid metabolism and restored hepatic morphology	HFD-induced diabetic mice	(Murakami et al. 2021)

1.11 Polysaccharides from Seaweeds

Seaweeds, which are also classified as marine macroalgae, are being recognized as valuable sources of numerous bioactive polymers and metabolites including polysaccharides with significant chemical and biological properties, thereby generating growing research interest in this area (Kumar Bhateja and Singh 2014). They are classified into three taxonomic groups based on their pigmentation, namely, green algae (Chlorophyta), red algae (Rhodophyta), and brown algae (Ochrophyta, Phaeophyceae) (Mohammadigheisar et al. 2020). More than 200 species of seaweeds are cultivated or collected from the wild for a variety of industries worldwide (Wade et al. 2020). A total of 32 green seaweeds, 64 brown seaweeds, and 125 red seaweeds are utilized economically (Pomin 2012). Out of 221 species of algae, 66% species are consumed as food, with 20% being green seaweeds, 26% being brown seaweeds, and 54% being red seaweeds (Pomin 2012; Wade et al. 2020). These consumptions have been common from time immemorial in China, Philippines and Indonesia, with Europe gaining interests in their application in culinary (Otero et al. 2023). In ethnomedicine, seaweeds have been explored for the treatment of various ailments including diabetes, obesity, cardiovascular diseases, and neurodegeneration (Olasehinde et al. 2019b; Olasehinde et al. 2020; Pillay et al. 2025). These medicinal properties have been attributed to the presence of diverse compounds such as phenolics, terpenes, alkaloids, peptides, vitamins and polysaccharides with diverse biological activities (Olasehinde et al. 2019b; Otero et al. 2023). Among these compounds, polysaccharides are the most the abundant in seaweeds as they account for over 75% of their dry weight and are mostly present as sulfated polysaccharides (Ciancia et al. 2020; Hentati et al. 2020). Their composition and structures differ among seaweed species, with green seaweeds rich in ulvan, red seaweeds rich in floridean starch, carrageenan, agar and

galactans, and brown seaweeds rich in laminarin, fucoidans, alginate and fucans (de Jesus Raposo et al. 2015; Hentati et al. 2020).

1.11.1 Red Seaweeds (*Gracilaria* species)

Commonly known red agar, the red seaweeds belong to the genus, *Gracilaria*. They are majorly found in Chile and China, as well as in other countries like Taiwan, South Africa, Namibia, the Philippines, and Vietnam (Pereira and Yarish 2008). They are bushy in appearance, with a cylindrically compressed and irregularly branched thallus (Pereira and Yarish 2008). They are the primary sources of agar and are mainly consumed in the Caribbean, Southeast Asia, Japan, and Hawaii (Khandwal et al. 2025). *Gracilaria* species have been reported for their polysaccharide constituents which consist mainly of floridean starch, carrageenan, agar and galactans. The medicinal properties of *Gracilaria* spp. polysaccharides have been reported to include antiinflammation, antioxidant, immunomodulation, neuroprotection, antidiabetes and antiobesity (Hentati et al. 2020; Olasehinde et al. 2019b; Pillay et al. 2024). These medicinal properties have been explored in the use of *Gracilaria* spp. for the management of diabetic hepatopathy.

There are limited studies on the therapeutic effect of *Gracilaria* spp. polysaccharides on diabetic hepatopathy. From literature search, only low molecular weight fucoidan, *Gracilaria lemaneiformis* and *Gracilaria lithophile* are the only *Gracilaria* spp. polysaccharides species that have been investigated for their protective effect on diabetic hepathopathy. As shown in Table 1.2, these *Gracilaria* spp. sulphated polysaccharides exhibit protective effect on diabetic hepatopathy by mitigating oxidative stress and inflammation, improving lipid and carbohydrate metabolism, and restoring tissue morphology (Huang et al. 2019; Liao et al. 2015; Lu et al. 2023; Wen et al. 2017; Zheng et al. 2018).

1.11.2 Brown Seaweeds (*Ecklonia* species)

Brown seaweeds belong to the class, Phaeophyceae and are among the major contributors to coastal environments' biomass (Silberfeld et al. 2010). They are rich in polyphenols, minerals, vitamins and polysaccharides, with the latter making 50-70% dry weight of the seaweeds (Li et al. 2021). Brown seaweed polysaccharides are highly sulfated and mainly consist of laminarin, fucoidans, alginate and fucans (de Jesus Raposo et al. 2015; Hentati et al. 2020). Brown seaweeds polysaccharides have been reported for their biological activities which include antiinflammation, antioxidant, anticancer, antiobesogenic, hepatoprotection and immunomodulation (Borazjani et al. 2018; Jiang et al. 2021; Murakami et al. 2021; Zhou et al. 2024). These biological activities have been explored in the use of brown seaweed polysaccharides in the treatment of diabetic hepatopathy as reported for *Laminaria japonica* Fucoidan (LJF) (Zhang et al. 2022) and *Sargassum horneri* (Murakami et al. 2021) (Table 1.2).

Among the species of brown seaweed is *Ecklonia maxima,* which is endemic to South Africa and has been reported for its rich content of sulphated polysaccharides (Pillay et al. 2025). *Ecklonia maxima* polysaccharides have been reported for their potent inhibition of glucose metabolizing enzymes and intestinal glucose absorption, as well as promotion of muscle glucose uptake (Daub et al. 2020; Pillay et al. 2025) which demonstrates its antidiabetic properties. Fucoidan EMLF7 extracted from *E. maxima* has been reported for its ability to inhibit inflammation in RAW 264.7 Macrophage by suppressing the production of (Prostaglandin) PGE2 and Nitric Oxide (NO), and downregulating TNF-α, IL-6, IL-1β and NF-κB/MAPK expressions (Lee et al. 2021; Nagahawatta et al. 2022). *Ecklonia maxima* polysaccharides have also been reported for their potent antioxidant and neuroprotective properties (Olasehinde et al. 2019a). Based on literature survey, there is a

dearth of information on the effect of *E. maxima* polysaccharides on diabetes and diabetic hepatopathy i*n vivo*.

1.12 Shelf Life

Shelf life refers to the storage time frame of a product when it can remain in acceptable condition in regards to safety, quality and sensory characteristics (Calligaris et al. 2019). It is dependent on the degradation mechanism of the product which is influenced by heat, light, enzymes, chemicals and microbial contamination (Azanha and Faria 2005). These influential features may simultaneously trigger degradation of a food product depending on the storage condition as summarized in Table 1.3.

The primary factors contributing to food degradation under chilled conditions are typically considered to be microbial growth and enzymatic reactions that negatively impact the sensory properties of the food (Mafe et al. 2024). However, microbial growths are not significant in food degradation under frozen conditions. Degradation of frozen foods are influenced by oxidative, enzymatic reactions, surface drying and re-crystallization (Calligaris et al. 2019). While at ambient conditions, food degradation often occurs from chemical reactions, such as lipid oxidation, flavor degradation, and pigment alteration, as well as non-enzymatic browning and physical changes including starch retrogradation and product structural collapse (Calligaris et al. 2019).

Table 1.3: Effect of storage conditions on degradative mechanism in food degradation

Storage conditions	Degradative events	References
Chilled	Microbial growth	(Erkmen and Bozoglu 2016)
	Enzymatic reactions	(Erkmen and Bozoglu 2016; Mafe et al. 2024)
	Senescence	(Heaton and Marangoni 1996; Mohammadi et al. 2021)
	Tissue mechanical damage	(Opara and Pathare 2024; Pathare and Al-Dairi 2022)
Frozen	Oxidative reactions	(Al-Dalali et al. 2022; Soyer et al. 2010)
	Enzymatic reactions	(Çalışkan Koç et al. 2025; Van der Sman 2020)
	Surface drying	(Schmidt and Lee 2009; Sun et al. 2023)
	Re-crystallization	(Ullah et al. 2014; Zhu et al. 2019)
Ambient	Oxidative reactions	(Geng et al. 2023; Wang et al. 2023)
	Non enzymatic browning	(Schutte et al. 2024; Yu et al. 2017)
	Caramelization	(Woo et al. 2015)
	Structural collapse	(Kawai and Hagiwara 2018; Oyinloye and Yoon 2020)
	Starch retrogradation	(Chen et al. 2024; Donmez et al. 2021)
	Packaging contaminants	(Gupta et al. 2024; Jadhav et al. 2021)

1.13 Shelf Life and Polysaccharide Degradation

The degradation of polysaccharides during shelf life has been implicated in changes in their texture and flavor, chemistry and biological activities (Feng et al. 2020; Peroni-Okita et al. 2013). The degradation of polysaccharides during shelf life is influenced by temperature and storage conditions, and occurs via various mechanisms such as retrogradation (Feng et al. 2020), enzymatic hydrolysis (Yu et al. 2022), freezing and thawing (Feng et al. 2020), cold-induced sweetening (Peroni-Okita et al. 2013; Yu et al. 2022), acid hydrolysis (Keran et al. 2025), thermal degradation (Silva et al. 2025) and UV irradiation (Song et al. 2024).

1.14 Shelf-Life Degradation and Biological Activities of Polysaccharides

The biological activities of polysaccharides are affected by shelf life degradation, leading to suppression of their effectiveness and changes in their chemistry (Xu et al. 2025; Zou et al. 2020). Improved antioxidant, monosaccharide compositions and shelf life were reported for *Dendrobium officinale* polysaccharides (Yu et al. 2019). Increased temperature also suppressed the antioxidant activity of *Pleurotus ostreatus* polysaccharides and altered its chemical compositions (Radzki et al. 2016). Preservation of polysaccharides with ascorbic acid has been reported to be influenced by temperatures and pH, with increased degradation reported for higher temperature and reducing pH (Zou et al. 2020).

Although shelf life degradation has been linked to reduced molecular weight of polysaccharides Zou et al. 2020), improved biological activities have been reported with reducing molecular weights (Song et al. 2024; Zhao et al. 2023; Zou et al. 2020). Furthermore, degradation products arising from shelf life process such as Maillard reaction, caramelization, and hydrolysis have also been reported for their potent antioxidant activities (Chen et al. 2019a; Ke and Li 2023). The

antioxidant and hypoglycemic properties of polysaccharides from blackcurrant fruits improved with reduced molecular weight following hydrolysis-mediated degradation (Xu et al. 2018). Aqueous hydrolysis improved the antitumor and antioxidant activities of pectic oligosaccharides (Li et al. 2019). Degradation improved the hypolipidemic and antioxidant activities of *Ganoderma lucidum* polysacharrides in hepatocytes (Xu et al. 2019). The ability of *Porphyra yezoensis* polysccahrrides to mitigate oxidative damage in renal epithelial cells was improved following degradation to small molecular weights (Sun et al. 2019).

1.15 Rationale of the study

Despite these studies, there is a dearth on the effect shelf life on the stability, chemical profiles and biological activities of sulphated polysaccharides from brown (*Ecklonia* species) and red seaweeds (*Gracilaria* species). The beneficial effects of these polysaccharides on diabetic hepatopathy are also limited. Therefore, it is important to study these effects over time and establish their translational relevance and safety in the management of hepatopathy in T2D.

1.15.1 Hypothesis

This study hypothesized that:

1. sulphated polysaccharides from seaweeds have a therapeutic effect on diabetic hepatopathy
2. the shelf life of sulphated polysaccharides from seaweeds at different storage conditions influences their antidiabetic and antioxidant activities, and profiles.

1.15.2 Aims

The study was aimed at determining the therapeutic effects of sulphated polysaccharides from *Ecklonia maxima* and *Gracilaria gracilis* on diabetic hepatopathy in T2D, as well as the effect of different storage conditions on their stability profile, antioxidant and antidiabetic activities.

1.15.3 Objectives

- To extract sulphated polysaccharides from *Ecklonia maxima* and *Gracilaria gracilis*
- To determine the stability of sulphated polysaccharides from *E. maxima* and *G. gracilis* seaweeds at different storage conditions over a period of time.
- To determine the effect of different storage conditions on glucose content, sulphate content and *in vitro* antidiabetic activities of the sulphated polysaccharides
- To determine the hepatoprotective effect of sulphated polysaccharide in T2D rats.

1.15.4 Research Questions

- Do sulphated polysaccharide from South African seaweeds have the potential to protect against diabetic hepatopathy in T2D?
- Will storage of sulphated polysaccharides from the seaweeds at different conditions affect their glucose and sulphate contents
- Will storage of sulphated polysaccharides from the seaweeds at different conditions affect their antidiabetic activities over time?

1.16. Reference

ADA (2015) 2. Classification and diagnosis of diabetes. Diabetes care 38(Supplement_1):S8-S16

Adeva-Andany MM, Pérez-Felpete N, Fernández-Fernández C, Donapetry-García C, Pazos-García C (2016) Liver glucose metabolism in humans. Bioscience reports 36(6):e00416

Ahmed N (2005) Advanced glycation endproducts—role in pathology of diabetic complications. Diabetes research and clinical practice 67(1):3-21

Åkerblom HK, Vaarala O, Hyöty H, Ilonen J, Knip M (2002) Environmental factors in the etiology of type 1 diabetes. American Journal of Medical Genetics Part A 115(1):18-29

Al-Dalali S, Li C, Xu B (2022) Effect of frozen storage on the lipid oxidation, protein oxidation, and flavor profile of marinated raw beef meat. Food Chemistry 376:131881

Antar SA, Ashour NA, Sharaky M, et al. (2023) Diabetes mellitus: Classification, mediators, and complications; A gate to identify potential targets for the development of new effective treatments. Biomedicine & Pharmacotherapy 168:115734

Aronson D (2008) Hyperglycemia and the pathobiology of diabetic complications Cardiovascular Diabetology: Clinical, Metabolic and Inflammatory Facets. vol 45. Karger Publishers, p 1-16

Azanha A, Faria J (2005) Use of mathematical models for estimating the shelf-life of cornflakes in flexible packaging. Packaging Technology and Science: An International Journal 18(4):171178

Bai Z, Huang X, Wu G, et al. (2023) Hepatic metabolism-related effects of polysaccharides from red kidney bean and small black soybean on type 2 diabetes. Food Chemistry 403:134334

Barb D, Repetto EM, Stokes ME, Shankar SS, Cusi K (2021) Type 2 diabetes mellitus increases the risk of hepatic fibrosis in individuals with obesity and nonalcoholic fatty liver disease. Obesity 29(11):1950-1960

Borazjani NJ, Tabarsa M, You S, Rezaei M (2018) Purification, molecular properties, structural characterization, and immunomodulatory activities of water soluble polysaccharides from Sargassum angustifolium. International journal of biological macromolecules 109:793-802

Bösenberg LH, van Zyl DG (2008) The mechanism of action of oral antidiabetic drugs: a review of recent literature. Journal of Endocrinology, Metabolism and Diabetes of South Africa 13(3):8088

Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414(6865):813

Brownlee M (2005) The pathobiology of diabetic complications: a unifying mechanism. Diabetes 54(6):1615-1625

Bulum T, Kolarić B, Duvnjak L, Duvnjak M (2011) Nonalcoholic fatty liver disease markers are associated with insulin resistance in type 1 diabetes. Digestive diseases and sciences 56:3655-3663

Bunner AE, Chandrasekera PC, Barnard ND (2014) Knockout mouse models of insulin signaling: Relevance past and future. World journal of diabetes 5(2):146

Burrack AL, Martinov T, Fife BT (2017) T cell-mediated beta cell destruction: autoimmunity and alloimmunity in the context of type 1 diabetes. Frontiers in endocrinology 8:343

Butalia S, Kaplan GG, Khokhar B, Rabi DM (2016) Environmental risk factors and type 1 diabetes: past, present, and future. Canadian journal of diabetes 40(6):586-593

Çalışkan Koç G, Özkan Karabacak A, Süfer Ö, et al. (2025) Thawing frozen foods: A comparative review of traditional and innovative methods. Comprehensive Reviews in Food Science and Food Safety 24(2):e70136

Calligaris S, Manzocco L, Anese M, Nicoli MC (2019) Accelerated shelf life testing Food quality and shelf life. Elsevier, p 359-392

Campbell I (2007) Oral antidiabetic drugs: their properties and recommended use. Prescriber 18(6):56-74

Carpentier AC (2021) 100th anniversary of the discovery of insulin perspective: insulin and adipose tissue fatty acid metabolism. American Journal of Physiology-Endocrinology and Metabolism 320(4):E653-E670

Catalfamo DL, Britten TM, Storch DI, Calderon NL, Sorenson HL, Wallet SM (2013)

Hyperglycemia induced and intrinsic alterations in type 2 diabetes-derived osteoclast function. Oral diseases 19(3):303-312

Cernea S, Dobreanu M (2013) Diabetes and beta cell function: from mechanisms to evaluation and clinical implications. Biochemia medica 23(3):266-280

Chang S-C, Yang W-CV (2016) Hyperglycemia, tumorigenesis, and chronic inflammation. Critical reviews in oncology/hematology 108:146-153

Chen K, Zhao J, Shi X, Abdul Q, Jiang Z (2019a) Characterization and antioxidant activity of products derived from xylose–bovine casein hydrolysate Maillard reaction: Impact of reaction time. Foods 8(7):242

Chen W, Xu W, Junejo SA, et al. (2024) Analytical methods for starch retrogradation assessment. Food Biomacromolecules 1(2):107-126

Chen X, Jin J, Tang J, et al. (2011) Extraction, purification, characterization and hypoglycemic activity of a polysaccharide isolated from the root of Ophiopogon japonicus. Carbohydrate Polymers 83(2):749-754

Chen Y, Liu D, Wang D, et al. (2019b) Hypoglycemic activity and gut microbiota regulation of a novel polysaccharide from Grifola frondosa in type 2 diabetic mice. Food and Chemical Toxicology 126:295-302

Chiacchiaretta P, Tumini S, Mascitelli A, et al. (2024) The Impact of Atmospheric Temperature Variations on Glycaemic Patterns in Children and Young Adults with Type 1 Diabetes. Climate 12(8):121

Chikezie PC, Ojiako OA, Ogbuji AC (2015) Oxidative stress in diabetes mellitus. Integrative Obesity and Diabetes 9(3):92-109

Chiniwala N, Jabbour S (2011) Management of diabetes mellitus in the elderly. Current Opinion in Endocrinology, Diabetes and Obesity 18(2):148-152

Ciancia M, Fernández PV, Leliaert F (2020) Diversity of sulfated polysaccharides from cell walls of coenocytic green algae and their structural relationships in view of green algal evolution. Frontiers in plant science 11:554585

Cucca F, Dudbridge F, Loddo M, et al. (2001) The HLA-DPB1–associated component of the IDDM1 and its relationship to the major loci HLA-DQB1,-DQA1, and-DRB1. Diabetes 50(5):1200-1205

Daub CD, Mabate B, Malgas S, Pletschke BI (2020) Fucoidan from Ecklonia maxima is a powerful inhibitor of the diabetes-related enzyme, α-glucosidase. International journal of biological macromolecules 151:412-420 de Jesus Raposo MF, De Morais AMB, De Morais RMSC (2015) Marine polysaccharides from algae with potential biomedical applications. Marine drugs 13(5):2967-3028 de la Monte SM, Wands JR (2008) Alzheimer's disease is type 3 diabetes—evidence reviewed. Journal of Diabetes Science and Technology 2(6):1101-1113

De Marco R, Locatelli F, Zoppini G, Verlato G, Bonora E, Muggeo M (1999) Cause-specific mortality in type 2 diabetes. The Verona Diabetes Study. Diabetes care 22(5):756-761

Defronzo RA (1997) Pathogenesis of type2 daiabetes: metabolic and molecular implications for identhfying diabetes genes. Diabetes Reviews 5:177-269

DeFronzo RA (2009) From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 58(4):773-795

DeFronzo RA (2018) Pathogenesis of Type 2 Diabetes Mellitus. In: Bonora E, DeFronzo RA (eds) Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment. Springer International Publishing, Cham, p 181-253

DeFronzo RA, Ferrannini E, Groop L, et al. (2015) Type 2 diabetes mellitus. Nature Reviews Disease Primers 1:15019

Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA (2011) Insulin effects in muscle and adipose tissue. Diabetes research and clinical practice 93:S52-S59

Donmez D, Pinho L, Patel B, Desam P, Campanella OH (2021) Characterization of starch–water interactions and their effects on two key functional properties: Starch gelatinization and retrogradation. Current Opinion in Food Science 39:103-109

Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M (2001) Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. Journal of Clinical Investigation 108(9):1341-1348

Eguchi N, Vaziri ND, Dafoe DC, Ichii H (2021) The role of oxidative stress in pancreatic β cell dysfunction in diabetes. International journal of molecular sciences 22(4):1509

El-Mir M-Y, Nogueira V, Fontaine E, Avéret N, Rigoulet M, Leverve X (2000) Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. Journal of Biological Chemistry 275(1):223-228

Erkmen O, Bozoglu TF (2016) Food preservation by low temperatures. Food Microbiology: Principles into Practice John Wiley and Sons, Ltd, Chichester, UK

Erukainure OL, Chukwuma CI (2024) African walnut (Plukenetia conophora) oil promotes glucose uptake while improving energy metabolism and steroidogenesis and maintaining surface architecture in rat testes. Frontiers in Nutrition 11:1505453

Erukainure OL, Hafizur R, Kabir N, et al. (2018) Suppressive Effects of Clerodendrum volubile P Beauv.[Labiatae] Methanolic Extract and Its Fractions on Type 2 Diabetes and Its Complications. Frontiers in Pharmacology 9:8 doi:10.3389/fphar.2018.00008

Erukainure OL, Mopuri R, Oyebode OA, Koorbanally NA, Islam MS (2017) Dacryodes edulis enhances antioxidant activities, suppresses DNA fragmentation in oxidative pancreatic and hepatic

injuries; and inhibits carbohydrate digestive enzymes linked to type 2 diabetes. Biomedicine & pharmacotherapy 96:37-47

Erukainure OL, Oyebode OA, Chuturgoon AA, et al. (2024) Potential molecular mechanisms underlying the ameliorative effect of Cola nitida (Vent.) Schott & Endl. on insulin resistance in rat skeletal muscles. Journal of Ethnopharmacology 319:117249

Erukainure OL, Oyebode OA, Ijomone OM, Chukwuma CI, Koorbanally NA, Islam MS (2019) Raffia palm (Raphia hookeri G. Mann & H. Wendl) wine modulates glucose homeostasis by enhancing insulin secretion and inhibiting redox imbalance in a rat model of diabetes induced by high fructose diet and streptozotocin. Journal of ethnopharmacology 237:159-170

Feng W, Ma S, Wang X (2020) Recent advances in quality deterioration and improvement of starch in frozen dough. Grain & Oil Science and Technology 3(4):154-163

Fernandes PA, Coimbra MA (2023) The antioxidant activity of polysaccharides: A structurefunction relationship overview. Carbohydrate Polymers 314:120965

Ganeshpurkar A, Kohli S, Rai G (2014) Antidiabetic potential of polysaccharides from the white oyster culinary-medicinal mushroom Pleurotus florida (higher Basidiomycetes). International Journal of Medicinal Mushrooms 16(3)

Garcia-Compean D, Jaquez-Quintana JO, Gonzalez-Gonzalez JA, Maldonado-Garza H (2009) Liver cirrhosis and diabetes: risk factors, pathophysiology, clinical implications and management. World journal of gastroenterology: WJG 15(3):280

Geng L, Liu K, Zhang H (2023) Lipid oxidation in foods and its implications on proteins. Frontiers in Nutrition 10:1192199

Geng T, Huang T (2020) Gene-environment interactions and type 2 diabetes. Asia Pacific Journal of Clinical Nutrition 29(2):220-226

Giordano S, Martocchia A, Toussan L, et al. (2014) Diagnosis of hepatic glycogenosis in poorly controlled type 1 diabetes mellitus. World journal of diabetes 5(6):882

Giri B, Dey S, Das T, Sarkar M, Banerjee J, Dash SK (2018) Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: An update on glucose toxicity. Biomedicine & pharmacotherapy 107:306-328

Giwa AM, Ahmed R, Omidian Z, et al. (2020) Current understandings of the pathogenesis of type 1 diabetes: Genetics to environment. World journal of diabetes 11(1):13

Goldin A, Beckman JA, Schmidt AM, Creager MA (2006) Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation 114(6):597-605

González P, Lozano P, Ros G, Solano F (2023) Hyperglycemia and oxidative stress: An integral, updated and critical overview of their metabolic interconnections. International Journal of Molecular Sciences 24(11):9352

Gupta RK, Pipliya S, Karunanithi S, et al. (2024) Migration of Chemical Compounds from Packaging Materials into Packaged Foods: Interaction, Mechanism, Assessment, and Regulations. Foods 13(19):3125

Gustafson B, Hedjazifar S, Gogg S, Hammarstedt A, Smith U (2015) Insulin resistance and impaired adipogenesis. Trends in Endocrinology & Metabolism 26(4):193-200

Hakonarson H, Grant SF, Bradfield JP, et al. (2007) A genome-wide association study identifies KIAA0350 as a type 1 diabetes gene. Nature 448(7153):591-594

Han H-S, Kang G, Kim JS, Choi BH, Koo S-H (2016) Regulation of glucose metabolism from a liver-centric perspective. Experimental & molecular medicine 48(3):e218-e218

Heaton JW, Marangoni AG (1996) Chlorophyll degradation in processed foods and senescent plant tissues. Trends in Food Science & Technology 7(1):8-15

Hentati F, Tounsi L, Djomdi D, et al. (2020) Bioactive polysaccharides from seaweeds. Molecules 25(14):3152

Holst JJ (2007) The physiology of glucagon-like peptide 1. Physiological reviews

Hong T, Zhao J, Dong M, Meng Y, Mu J, Yang Z (2012) Composition and bioactivity of polysaccharides from Inula britannica flower. International Journal of Biological Macromolecules 51(4):550-554

Hotamisligil G (2000) Molecular mechanisms of insulin resistance and the role of the adipocyte. International Journal of Obesity 24(4):S23-S27

Houeiss P, Luce S, Boitard C (2022) Environmental triggering of type 1 diabetes autoimmunity. Frontiers in Endocrinology 13:933965

Hu J, Pang W, Chen J, Bai S, Zheng Z, Wu X (2013a) Hypoglycemic effect of polysaccharides with different molecular weight of Pseudostellaria heterophylla. BMC Complementary and Alternative Medicine 13:1-9

Hu S, Chang Y, Wang J, Xue C, Li Z, Wang Y (2013b) Fucosylated chondroitin sulfate from sea cucumber in combination with rosiglitazone improved glucose metabolism in the liver of the insulin-resistant mice. Bioscience, Biotechnology, and Biochemistry 77(11):2263-2268 Hu S, Chang Y, Wang J, et al. (2013c) Fucosylated chondroitin sulfate from Acaudina molpadioides improves hyperglycemia via activation of PKB/GLUT4 signaling in skeletal muscle of insulin resistant mice. Food & Function 4(11):1639-1646

Hu S, Wang J, Xu H, Wang Y, Li Z, Xue C (2014a) Fucosylated chondroitin sulphate from sea cucumber inhibits high-fat-sucrose diet-induced apoptosis in mouse pancreatic islets via downregulating mitochondrial signaling pathway. Journal of Functional Foods 7:517-526

Hu S, Xu H, Chen R, Wang J, Li Z, Xu J (2014b) Activation of PKB and ERK, but not PI3K, is involved in fucosylated chondroitin sulphate from Acaudina molpadioides induced glucose uptake. Journal of Functional Foods 10:385-396

Huang M, Wang F, Zhou X, Yang H, Wang Y (2015) Hypoglycemic and hypolipidemic properties of polysaccharides from Enterobacter cloacae Z0206 in KKAy mice. Carbohydrate polymers 117:91-98

Huang S, Pang D, Li X, et al. (2019) A sulfated polysaccharide from Gracilaria Lemaneiformis regulates cholesterol and bile acid metabolism in high-fat diet mice. Food & Function 10(6):32243236

Huang Z, Hu M, Peng X, Wang R, Song X, Yin J (2023) The protective effect of small black soybean (Vigna Mungo L.) polysaccharide on acetic acid-induced gastric ulcer in SD rats and its impact on gut microbiota and metabolites. Food Bioscience 56:103187

IDF (2021) IDF diabetes atlas. In. https://diabetesatlas.org/idfawp/resourcefiles/2021/07/IDF_Atlas_10th_Edition_2021.pdf Accessed July 14, 2023 2023

Im S-S, Kang S-Y, Kim S-Y, et al. (2005) Glucose-stimulated upregulation of GLUT2 gene is mediated by sterol response element–binding protein-1c in the hepatocytes. Diabetes 54(6):16841691

Inoguchi T, Li P, Umeda F, et al. (2000) High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent activation of NAD (P) H oxidase in cultured vascular cells. Diabetes 49(11):1939-1945

Islam S, Rahman S, Haque T, Sumon AH, Ahmed AM, Ali N (2020) Prevalence of elevated liver enzymes and its association with type 2 diabetes: A cross-sectional study in Bangladeshi adults. Endocrinology, diabetes & metabolism 3(2):e00116

Iynedjian P, Marie S, Gjinovci A, et al. (1995) Glucokinase and cytosolic phosphoenolpyruvate carboxykinase (GTP) in the human liver. Regulation of gene expression in cultured hepatocytes. The Journal of clinical investigation 95(5):1966-1973

Jadhav EB, Sankhla MS, Bhat RA, Bhagat D (2021) Microplastics from food packaging: An overview of human consumption, health threats, and alternative solutions. Environmental Nanotechnology, Monitoring & Management 16:100608

Jahromi MM, Eisenbarth GS (2007) Cellular and molecular pathogenesis of type 1A diabetes. Cellular and Molecular Life Sciences 64(7-8):865-872

Jiang H, Yang SQ, Chakka VP, et al. (2021) Purification and biological activities of enzymatically degraded Sargassum fusiforme polysaccharides. Chemistry & Biodiversity 18(3):e2000930

Jin H, Zhang Y-J, Jiang J-X, et al. (2013) Studies on the extraction of pumpkin components and their biological effects on blood glucose of diabetic mice. Journal of food and drug analysis 21(2):184-189

Kajimoto Y, Kaneto H (2004) Role of Oxidative Stress in Pancreatic β-Cell Dysfunction. Annals of the New York Academy of Sciences 1011(1):168-176

Kanagasabapathy G, Kuppusamy UR, Abd Malek SN, Abdulla MA, Chua K-H, Sabaratnam V (2012) Glucan-rich polysaccharides from Pleurotus sajor-caju (Fr.) Singer prevents glucose intolerance, insulin resistance and inflammation in C57BL/6J mice fed a high-fat diet. BMC Complementary and Alternative Medicine 12:1-9

Kaul N, Ali S (2016) Genes, genetics, and environment in type 2 diabetes: implication in personalized medicine. DNA and Cell Biology 35(1):1-12

Kawai K, Hagiwara T (2018) Control of physical changes in food products. Survival strategies in extreme cold and desiccation: Adaptation mechanisms and their applications:385-399

Ke C, Li L (2023) Influence mechanism of polysaccharides induced Maillard reaction on plant proteins structure and functional properties: A review. Carbohydrate Polymers 302:120430

Ke Y, Yang J, Lin H Advances in the structural characterization and pharmacological activity of Salvia miltiorrhiza polysaccharides. Frontiers in Chemistry 13:1492533

Keran DA, Kumar PS, Venugopal V, Kumar R, Tarakanth K, Chinnappa A (2025) Degradation of polysaccharides during food processing Chemistry of Thermal and Non-Thermal Food Processing Technologies. Elsevier, p 461-501

Kerner W, Brückel J (2014) Definition, classification and diagnosis of diabetes mellitus. Experimental and clinical endocrinology & diabetes 122(07):384-386

Khandwal D, Patel S, Pandey AK, Mishra A (2025) A Comprehensive, Analytical Narrative Review of Polysaccharides from the Red Seaweed Gracilaria: Pharmaceutical Applications and Mechanistic Insights for Human Health. Nutrients 17(5):744

Kim MS, Polychronakos C (2005) Immunogenetics of type 1 diabetes. Hormone Research in Paediatrics 64(4):180-188

Knip M, Virtanen SM, Åkerblom HK (2010) Infant feeding and the risk of type 1 diabetes. The American journal of clinical nutrition 91(5):1506S-1513S

Kumar Bhateja P, Singh R (2014) Antidiabetic Activity of Acacia Tortilis (Forsk.) Hayne Ssp. Raddiana Polysaccharide on Streptozotocin-Nicotinamide Induced Diabetic Rats. BioMed research international 2014(1):572013

Lee H-G, Je J-G, Hwang J, et al. (2021) Comparision of antioxidant and anti-inflammatory activities of enzyme assisted hydrolysate from Ecklonia maxima blades and stipe. Fisheries and Aquatic Sciences 24(5):197-206

Leszek J, Trypka E, V Tarasov V, Md Ashraf G, Aliev G (2017) Type 3 diabetes mellitus: a novel implication of Alzheimers disease. Current Topics in Medicinal Chemistry 17(12):1331-1335 Li F, Zhang Y, Zhong Z (2011) Antihyperglycemic effect of Ganoderma lucidum polysaccharides on streptozotocin-induced diabetic mice. International journal of molecular sciences 12(9):61356145

Li HN, Zhao LL, Chen DQ (2020) Ganoderma lucidum polysaccharides ameliorates hepatic steatosis and oxidative stress in db/db mice via targeting nuclear factor E2 (erythroid-derived 2)related factor-2/heme oxygenase-1 (HO-1) pathway. Medical Science Monitor: international medical journal of experimental and clinical research 26:e921905-1

Li J, Li S, Liu S, et al. (2019) Pectic oligosaccharides hydrolyzed from citrus canning processing water by Fenton reaction and their antiproliferation potentials. International journal of biological macromolecules 124:1025-1032

Li S, Chen H, Wang J, Wang X, Hu B, Lv F (2015) Involvement of the PI3K/Akt signal pathway in the hypoglycemic effects of tea polysaccharides on diabetic mice. International Journal of Biological Macromolecules 81:967-974

Li Y, Liu Y, Liu S, et al. (2023) Diabetic vascular diseases: molecular mechanisms and therapeutic strategies. Signal transduction and targeted therapy 8(1):152

Li Y, Zheng Y, Zhang Y, et al. (2021) Brown algae carbohydrates: Structures, pharmaceutical properties, and research challenges. Marine drugs 19(11):620

Liao X, Yang L, Chen M, Yu J, Zhang S, Ju Y (2015) The hypoglycemic effect of a polysaccharide (GLP) from Gracilaria lemaneiformis and its degradation products in diabetic mice. Food & function 6(8):2542-2549

Lie BA, Todd JA, Pociot F, et al. (1999) The predisposition to type 1 diabetes linked to the human leukocyte antigen complex includes at least one non–class II gene. The American Journal of Human Genetics 64(3):793-800

Lin W, Wang W, Liao D, et al. (2015) Polysaccharides from Enteromorpha prolifera improve glucose metabolism in diabetic rats. Journal of Diabetes Research 2015(1):675201

Liu C-M, Ma J-Q, Sun Y-Z (2012) Puerarin protects rat kidney from lead-induced apoptosis by modulating the PI3K/Akt/eNOS pathway. Toxicology and Applied Pharmacology 258(3):330-342

Liu Y, Sun J, Rao S, et al. (2013a) Antidiabetic activity of mycelia selenium-polysaccharide from Catathelasma ventricosum in STZ-induced diabetic mice. Food and Chemical Toxicology 62:285291

Liu Y, Sun J, Rao S, Su Y, Yang Y (2013b) Antihyperglycemic, antihyperlipidemic and antioxidant activities of polysaccharides from Catathelasma ventricosum in streptozotocininduced diabetic mice. Food and Chemical Toxicology 57:39-45

Liu Y, Wan L, Xiao Z, Wang J, Wang Y, Chen J (2013c) Antidiabetic activity of polysaccharides from tuberous root of Liriope spicata var. prolifera in KKAy mice. Evidence-Based Complementary and Alternative Medicine 2013(1):349790

Liu Y, Yang L, Zhang Y, et al. (2020) Dendrobium officinale polysaccharide ameliorates diabetic hepatic glucose metabolism via glucagon-mediated signaling pathways and modifying liverglycogen structure. Journal of Ethnopharmacology 248:112308

Lovegrove A, Edwards C, De Noni I, et al. (2017) Role of polysaccharides in food, digestion, and health. Critical Reviews in Food Science and Nutrition 57(2):237-253

Lu S-Y, Tan K, Zhong S, Cheong K-L (2023) Marine algal polysaccharides as future potential constituents against non-alcoholic steatohepatitis. International Journal of Biological Macromolecules 250:126247

Luo X, Wu J, Jing S, Yan L-J (2016) Hyperglycemic stress and carbon stress in diabetic glucotoxicity. Aging and Disease 7(1):90-110

Ma X, Zhou F, Chen Y, et al. (2014) A polysaccharide from Grifola frondosa relieves insulin resistance of HepG2 cell by Akt-GSK-3 pathway. Glycoconjugate journal 31:355-363

Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ (2010) Epidemiology of type 1 diabetes. Endocrinology and Metabolism Clinics 39(3):481-497

Madiraju AK, Erion DM, Rahimi Y, et al. (2014) Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature 510(7506):542-546

Mafe AN, Edo GI, Makia RS, et al. (2024) A review on food spoilage mechanisms, food borne diseases and commercial aspects of food preservation and processing. Food Chemistry Advances 5:100852

Mallet V, Parlati L, Martinino A, et al. (2022) Burden of liver disease progression in hospitalized patients with type 2 diabetes mellitus. Journal of Hepatology 76(2):265-274

Martin M, Marais R (2012) Metformin: a diabetes drug for cancer, or a cancer drug for diabetics? Journal of Clinical Oncology 30(21):2698-2700

Mayerson AB, Hundal RS, Dufour S, et al. (2002) The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. Diabetes 51(3):797-802

Mba CM, Mbanya JC (2020) Challenges and economic burden of diabetes in Africa Obesity and Diabetes: Scientific Advances and Best Practice. Springer, p 21-34

Meyerstein D (2021) Re-examining Fenton and Fenton-like reactions. Nature Reviews Chemistry 5(9):595-597

Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ (2013) Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature 494(7436):256-260

Mobasheri L, Ahadi M, Namdar AB, et al. (2023) Pathophysiology of diabetic hepatopathy and molecular mechanisms underlying the hepatoprotective effects of phytochemicals. Biomedicine & Pharmacotherapy 167:115502

Mohammadi M, Aelaei M, Saidi M (2021) Pre-harvest spray of GABA and spermine delays postharvest senescence and alleviates chilling injury of gerbera cut flowers during cold storage. Scientific Reports 11(1):14166

Mohammadigheisar M, Shouldice V, Sands J, Lepp D, Diarra M, Kiarie E (2020) Growth performance, breast yield, gastrointestinal ecology and plasma biochemical profile in broiler chickens fed multiple doses of a blend of red, brown and green seaweeds. British Poultry Science 61(5):590-598

Mooradian AD (1988) Diabetic complications of the central nervous system. Endocrine Reviews 9(3):346-356

Mphasha MH, Stubbendorff A (2025) Challenging Diabetes in Rural Areas: Aspects of Nutrition Handbook of Public Health Nutrition: International, National, and Regional Perspectives. Springer, p 1-23

Msomi NZ, Erukainure OL, Salau VF, Olofinsan KA, Islam MS (2023) 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. Food Science and Human Wellness 12(6):2052-2060

Murakami S, Hirazawa C, Ohya T, et al. (2021) The edible brown seaweed Sargassum horneri (Turner) C. Agardh ameliorates high-fat diet-induced obesity, diabetes, and hepatic steatosis in mice. Nutrients 13(2):551

Murea M, Ma L, Freedman BI (2012) Genetic and environmental factors associated with type 2 diabetes and diabetic vascular complications. Review of Diabetic Studies 9(1):6

Nagahawatta D, Liyanage N, Jayawardhana H, Lee H-G, Jayawardena TU, Jeon Y-J (2022) Antifine dust effect of fucoidan extracted from Ecklonia maxima leaves in macrophages via inhibiting inflammatory signaling pathways. Marine Drugs 20(7):413

Noble JA, Valdes AM (2011) Genetics of the HLA region in the prediction of type 1 diabetes. Current diabetes reports 11:533-542

Olasehinde TA, Mabinya LV, Olaniran AO, Okoh AI (2019a) Chemical characterization, antioxidant properties, cholinesterase inhibitory and anti-amyloidogenic activities of sulfated polysaccharides from some seaweeds. Bioactive Carbohydrates and Dietary Fibre 18:100182

Olasehinde TA, Olaniran AO, Okoh AI (2019b) Macroalgae as a valuable source of naturally occurring bioactive compounds for the treatment of Alzheimer's disease. Marine drugs 17(11):609

Olasehinde TA, Olaniran AO, Okoh AI (2020) Sulfated polysaccharides of some seaweeds exhibit neuroprotection via mitigation of oxidative stress, cholinergic dysfunction and inhibition of Zn–induced neuronal damage in HT-22 cells. BMC complementary medicine and therapies 20:1-10

Opara UL, Pathare PB (2024) Mechanical damage of fresh produce: An overview. Mechanical Damage in Fresh Horticultural Produce: Measurement, Analysis and Control:1-19

Osadebe PO, Odoh EU, Uzor PF (2015) Oral Anti-Diabetic Agents-Review and Updates. British Journal of Medicine & Medical Research 5(2):134-159

Otero P, Carpena M, Garcia-Oliveira P, et al. (2023) Seaweed polysaccharides: Emerging extraction technologies, chemical modifications and bioactive properties. Critical Reviews in Food Science and Nutrition 63(13):1901-1929

Owen MR, Doran E, Halestrap AP (2000) Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochemical journal 348(3):607-614

Oyinloye TM, Yoon WB (2020) Effect of freeze-drying on quality and grinding process of food produce: A review. Processes 8(3):354

Ozougwu J, Obimba K, Belonwu C, Unakalamba C (2013) The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pathophysiol 4(4):46-57

Pan D, Wang L, Chen C, Hu B, Zhou P (2015) Isolation and characterization of a hyperbranched proteoglycan from Ganoderma lucidum for anti-diabetes. Carbohydrate Polymers 117:106-114

Pathare PB, Al-Dairi M (2022) Effect of mechanical damage on the quality characteristics of banana fruits during short-term storage. Discover Food 2(1):4

Pereira R, Yarish C (2008) Mass production of marine macroalgae In: Jørgensen, SE, Fath, BD (Eds.), Encyclopedia of Ecology. Academic Press, Oxford

Perkins RM, Yuan CM, Welch PG (2006) Dipsogenic diabetes insipidus: report of a novel treatment strategy and literature review. Clinical and Experimental Nephrology 10(1):63-67

Peroni-Okita FH, Cardoso MB, Agopian RG, et al. (2013) The cold storage of green bananas affects the starch degradation during ripening at higher temperature. Carbohydrate polymers 96(1):137-147

Petersen MC, Shulman GI (2018) Mechanisms of insulin action and insulin resistance. Physiological Reviews

Pi J, Zhang Q, Fu J, et al. (2010) ROS signaling, oxidative stress and Nrf2 in pancreatic beta-cell function. Toxicology and Applied Pharmacology 244(1):77-83

Pillay LR, Olasehinde TA, Olofinsan K, et al. (2025) The impact of sulphated polysaccharides from Ecklonia maxima on carbohydrate hydrolyzing enzymes, muscle glucose uptake and intestinal glucose absorption ex vivo. Algal Research 85:103831

Pillay LR, Olasehinde TA, Olofinsan KA, Erukainure OL, Islam MS, Olaniran AO (2024) Antidiabetic potentials of crude and purified sulphated polysaccharides isolated from Gracilaria gracilis, a seaweed from South Africa. Heliyon 10(15)

Pirot P, Cardozo AK, Eizirik DL (2008) Mediators and mechanisms of pancreatic beta-cell death in type 1 diabetes. Arquivos Brasileiros de Endocrinologia & Metabologia 52(2):156-165

Poitout V, Robertson RP (2008) Glucolipotoxicity: fuel excess and β-cell dysfunction. Endocrine reviews 29(3):351-366

Pomin VH (2012) Seaweed: Ecology, nutrient composition and medicinal uses. (No Title)

Ponce NM, Stortz CA (2020) A comprehensive and comparative analysis of the fucoidan compositional data across the Phaeophyceae. Frontiers in Plant Science 11:556312

Pratley RE, Salsali A (2007) Inhibition of DPP-4: a new therapeutic approach for the treatment of type 2 diabetes. Current Medical Research and Opinion 23(4):919-931

Qaid MM, Abdelrahman MM (2016) Role of insulin and other related hormones in energy metabolism—A review. Cogent Food & Agriculture 2(1):1267691 doi:10.1080/23311932.2016.1267691

Radzki W, Ziaja-Sołtys M, Nowak J, et al. (2016) Effect of processing on the content and biological activity of polysaccharides from Pleurotus ostreatus mushroom. LWT-Food Science and Technology 66:27-33

Ren C, Zhang Y, Cui W, et al. (2015) A polysaccharide extract of mulberry leaf ameliorates hepatic glucose metabolism and insulin signaling in rats with type 2 diabetes induced by high fat-diet and streptozotocin. International journal of biological macromolecules 72:951-959

Ren D, Zhao Y, Nie Y, Yang N, Yang X (2014) Hypoglycemic and hepatoprotective effects of polysaccharides from Artemisia sphaerocephala Krasch seeds. International journal of biological macromolecules 69:296-306

Rolo AP, Palmeira CM (2006) Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. Toxicology and Applied Pharmacology 212(2):167-178

Saborio P, Tipton GA, Chan J (2000) Diabetes insipidus. Pediatrics in Review 21(4):122-129

Schmidt SJ, Lee JW (2009) How does the freezer burn our food? Journal of Food Science Education 8(2):45-52

Schutte M, Hayward S, Manley M (2024) Nonenzymatic browning and antioxidant properties of thermally treated cereal grains and end products. Journal of Food Biochemistry 2024(1):3865849

Sharabi K, Tavares CD, Rines AK, Puigserver P (2015) Molecular pathophysiology of hepatic glucose production. Molecular aspects of medicine 46:21-33

Shaw K (2017) ‘Type 3’diabetes: a brain insulin-resistant state linked to Alzheimer's disease. Practical Diabetes 34(6):187-188

Silberfeld T, Leigh JW, Verbruggen H, Cruaud C, De Reviers B, Rousseau F (2010) A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae):

Investigating the evolutionary nature of the “brown algal crown radiation”. Molecular phylogenetics and evolution 56(2):659-674

Silva M, Vida M, Ramos AC, Lidon FJ, Reboredo FH, Gonçalves EM (2025) Storage Temperature Effect on Quality and Shelf-Life of Hericium erinaceus Mushroom. Horticulturae 11(2):158

Singh RP (2024) Kidney bean starch: composition, structure, properties, and modifications Non-Conventional Starch Sources. Elsevier, p 439-465

Song P, Huang Y, Li J, et al. (2024) The influence of processing technologies on the biological activity of carbohydrates in food. Food Chemistry: X 23:101590

Soyer A, Özalp B, Dalmış Ü, Bilgin V (2010) Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. Food chemistry 120(4):1025-1030

Stankov K, Benc D, Draskovic D (2013) Genetic and epigenetic factors in etiology of diabetes mellitus type 1. Pediatrics 132(6):1112-1122

Sun C, Chen Y, Li X, Tai G, Fan Y, Zhou Y (2014) Anti-hyperglycemic and anti-oxidative activities of ginseng polysaccharides in STZ-induced diabetic mice. Food & Function 5(5):845848

Sun L, Zhu Z, Sun D-W (2023) Regulating ice formation for enhancing frozen food quality: Materials, mechanisms and challenges. Trends in Food Science & Technology 139:104116

Sun X-Y, Zhang H, Liu J, Ouyang J-M (2019) Repair activity and crystal adhesion inhibition of polysaccharides with different molecular weights from red algae Porphyra yezoensis against oxalate-induced oxidative damage in renal epithelial cells. Food & Function 10(7):3851-3867

Suzanne M (2014) Type 3 diabetes is sporadic Alzheimer′ s disease: Mini-review. European Neuropsychopharmacology 24(12):1954-1960

Suzuki A, Lindor K, St Saver J, et al. (2005) Effect of changes on body weight and lifestyle in nonalcoholic fatty liver disease. Journal of hepatology 43(6):1060-1066

Szablewski L (2014) Role of immune system in type 1 diabetes mellitus pathogenesis. International immunopharmacology 22(1):182-191

Thrasher J (2017) Pharmacologic management of type 2 diabetes mellitus: available therapies. American Journal of Cardiology 120(1):S4-S16

Tolman KG, Fonseca V, Dalpiaz A, Tan MH (2007) Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. Diabetes care 30(3):734-743

Tripathi BK, Srivastava AK (2006) Diabetes mellitus: Complications and therapeutics. Medical Science Monitor 12(7):RA130-RA147

Ullah J, Takhar PS, Sablani SS (2014) Effect of temperature fluctuations on ice-crystal growth in frozen potatoes during storage. LWT-Food Science and Technology 59(2):1186-1190

Utzschneider KM, Kahn SE (2006) The role of insulin resistance in nonalcoholic fatty liver disease. The Journal of Clinical Endocrinology & Metabolism 91(12):4753-4761

Vallon V, Nakagawa T (2021) Renal tubular handling of glucose and fructose in health and disease. Comprehensive Physiology 12(1):2995

Vallon V, Thomson SC (2017) Targeting renal glucose reabsorption to treat hyperglycaemia: the pleiotropic effects of SGLT2 inhibition. Diabetologia 60(2):215-225

Van der Sman R (2020) Impact of processing factors on quality of frozen vegetables and fruits. Food Engineering Reviews 12:399-420

Vilas-Boas EA, Almeida DC, Roma LP, Ortis F, Carpinelli AR (2021) Lipotoxicity and β-cell failure in type 2 diabetes: Oxidative stress linked to NADPH oxidase and ER stress. Cells 10(12):3328

Wade R, Augyte S, Harden M, Nuzhdin S, Yarish C, Alberto F (2020) Macroalgal germplasm banking for conservation, food security, and industry. PLoS biology 18(2):e3000641

Wang C, Gao X, Santhanam RK, et al. (2018) Effects of polysaccharides from Inonotus obliquus and its chromium (III) complex on advanced glycation end-products formation, α-amylase, αglucosidase activity and H2O2-induced oxidative damage in hepatic L02 cells. Food and Chemical Toxicology 116:335-345

Wang D, Xiao H, Lyu X, Chen H, Wei F (2023) Lipid oxidation in food science and nutritional health: A comprehensive review. Oil Crop Science 8(1):35-44

Wang F, Yang H, Wang Y (2013a) Structure characterization of a fucose-containing exopolysaccharide produced by Enterobacter cloacae Z0206. Carbohydrate polymers 92(1):503509

Wang J, Jin W, Zhang W, Hou Y, Zhang H, Zhang Q (2013b) Hypoglycemic property of acidic polysaccharide extracted from Saccharina japonica and its potential mechanism. Carbohydrate polymers 95(1):143-147

Wang L, Wu H, Chang N, Zhang K (2011) Anti-hyperglycemic effect of the polysaccharide fraction isolated from mactra veneriformis. Frontiers of Chemical Science and Engineering 5:238244

Wang L-Y, Wang Y, Xu D-S, Ruan K-F, Feng Y, Wang S (2012) MDG-1, a polysaccharide from Ophiopogon japonicus exerts hypoglycemic effects through the PI3K/Akt pathway in a diabetic KKAy mouse model. Journal of Ethnopharmacology 143(1):347-354

Wang Y (2017) Study on standardization method of Astragalus polysaccharide reference substance. Zhongcaoyao:4897-4903

Wei Z, Weng S, Wang L, Mao Z (2018) Mechanism of Astragalus polysaccharides in attenuating insulin resistance in Rats with type 2 diabetes mellitus via the regulation of liver microRNA-203a-3p. Molecular Medicine Reports 17(1):1617-1624

Wen L, Zhang Y, Sun-Waterhouse D, You L, Fu X (2017) Advantages of the polysaccharides from Gracilaria lemaneiformis over metformin in antidiabetic effects on streptozotocin-induced diabetic mice. RSC advances 7(15):9141-9151

Wilcox G (2005) Insulin and insulin resistance. Clinical Biochemist Reviews 26(2):19-39

Williams R, Karuranga S, Malanda B, et al. (2020) Global and regional estimates and projections of diabetes-related health expenditure: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Research and Clinical Practice 162:108072

Woo KS, Kim HY, Hwang IG, Lee SH, Jeong HS (2015) Characteristics of the thermal degradation of glucose and maltose solutions. Preventive nutrition and food science 20(2):102 Wu J, Shi S, Wang H, Wang S (2016) Mechanisms underlying the effect of polysaccharides in the treatment of type 2 diabetes: A review. Carbohydrate polymers 144:474-494

Xiang Y, Zhang M, Jiang D, Su Q, Shi J (2023) The role of inflammation in autoimmune disease: a therapeutic target. Frontiers in Immunology 14:1267091

Xiao C, Wu Q, Xie Y, Zhang J, Tan J (2015) Hypoglycemic effects of Grifola frondosa (Maitake) polysaccharides F2 and F3 through improvement of insulin resistance in diabetic rats. Food & function 6(11):3567-3575

Xiao C, Wu Q-P, Cai W, Tan J-B, Yang X-B, Zhang J-M (2012) Hypoglycemic effects of Ganoderma lucidum polysaccharides in type 2 diabetic mice. Archives of pharmacal research 35:1793-1801

Xiao X, Erukainure OL, Islam MS (2023) Red honeybush (Cyclopia genistoides) tea mitigates oxidative imbalance and hyperlipidemia, while improving glucose homeostasis in type 2 diabetic rats. Food Science and Human Wellness 12(6):2029-2039

Xiao Zq, Wang Yl, Gan Sr, Chen Jc (2014) Polysaccharides from Liriopes Radix ameliorates hyperglycemia via various potential mechanisms in diabetic rats. Journal of the Science of Food and Agriculture 94(5):975-982

Xu Bw, Li Ss, Ding Wl, et al. (2025) From structure to function: A comprehensive overview of polysaccharide roles and applications. Food Frontiers 6(1):15-39

Xu L, Yang F, Wang J, Huang H, Huang Y (2015a) Anti-diabetic effect mediated by Ramulus mori polysaccharides. Carbohydrate Polymers 117:63-69

Xu W, Zhou Q, Yin J-j, Yao Y, Zhang J-l (2015b) Anti-diabetic effects of polysaccharides from Talinum triangulare in streptozotocin (STZ)-induced type 2 diabetic male mice. International Journal of Biological Macromolecules 72:575-579

Xu Y, Niu X, Liu N, et al. (2018) Characterization, antioxidant and hypoglycemic activities of degraded polysaccharides from blackcurrant (Ribes nigrum L.) fruits. Food Chemistry 243:26-35

Xu Y, Zhang X, Yan X-H, et al. (2019) Characterization, hypolipidemic and antioxidant activities of degraded polysaccharides from Ganoderma lucidum. International journal of biological macromolecules 135:706-716

Yang X, Li A, Li X, Sun L, Guo Y (2020) An overview of classifications, properties of food polysaccharides and their links to applications in improving food textures. Trends in Food Science & Technology 102:1-15

Ye M, Qiu T, Peng W, Chen W-x, Ye Y-w, Lin Y-r (2011) Purification, characterization and hypoglycemic activity of extracellular polysaccharides from Lachnum calyculiforme. Carbohydrate Polymers 86(1):285-290

Yu A-N, Li Y, Yang Y, Yu K (2017) The browning kinetics of the non-enzymatic browning reaction in L-ascorbic acid/basic amino acid systems. Food Science and Technology 38:537-542 Yu J,

Tseng Y, Pham K, Liu M, Beckles DM (2022) Starch and sugars as determinants of postharvest shelf life and quality: some new and surprising roles. Current opinion in biotechnology 78:102844

Yu JG, Javorschi S, Hevener AL, et al. (2002) The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. Diabetes 51(10):2968-2974 Yu Z, Yang Z, da Silva JAT, Luo J, Duan J (2019) Influence of low temperature on physiology and bioactivity of postharvest Dendrobium officinale stems. Postharvest Biology and Technology 148:97-106

Zhang C, Jia J, Zhang P, et al. (2022) Fucoidan from Laminaria japonica ameliorates type 2 diabetes mellitus in association with modulation of gut microbiota and metabolites in streptozocintreated mice. Foods 12(1):33

Zhang J, Meng G, Zhang C, et al. (2015a) The antioxidative effects of acidic-, alkalic-, and enzymatic-extractable mycelium zinc polysaccharides by Pleurotus djamor on liver and kidney of streptozocin-induced diabetic mice. BMC complementary and alternative medicine 15:1-12

Zhang J-G, Liu Q, Liu Z-L, Li L, Yi L-t (2015b) Antihyperglycemic activity of Anoectochilus roxburghii polysaccharose in diabetic mice induced by high-fat diet and streptozotocin. Journal of Ethnopharmacology 164:180-185

Zhang L, Liu Y, Ke Y, et al. (2018) Antidiabetic activity of polysaccharides from Suillellus luridus in streptozotocin-induced diabetic mice. International journal of biological macromolecules 119:134-140

Zhang W, Zheng L, Zhang Z, Hai C-X (2012) Protective effect of a water-soluble polysaccharide from Salvia miltiorrhiza Bunge on insulin resistance in rats. Carbohydrate Polymers 89(3):890898

Zhang Y, Ren C, Lu G, et al. (2014) Purification, characterization and anti-diabetic activity of a polysaccharide from mulberry leaf. Regulatory Toxicology and Pharmacology 70(3):687-695

Zhao L, Lan Q, Huang Z, Ouyang L, Zeng F (2011) Antidiabetic effect of a newly identified component of Opuntia dillenii polysaccharides. Phytomedicine 18(8-9):661-668

Zhao R, Qiu B, Li Q, et al. (2014) LBP-4a improves insulin resistance via translocation and activation of GLUT4 in OLETF rats. Food & Function 5(4):811-820

Zhao T, Mao G-h, Zhang M, et al. (2013) Anti-diabetic effects of polysaccharides from ethanolinsoluble residue of Schisandra chinensis (Turcz.) Baill on alloxan-induced diabetic mice. chemical research in chinese universities 29:99-102

Zhao Z, Wang L, Ruan Y, et al. (2023) Physicochemical properties and biological activities of polysaccharides from the peel of Dioscorea opposita Thunb. extracted by four different methods. Food Science and Human Wellness 12(1):130-139

Zheng J, Yang B, Yu Y, Chen Q, Huang T, Li D (2012) Ganoderma lucidum polysaccharides exert anti-hyperglycemic effect on streptozotocin-induced diabetic rats through affecting β-cells. Combinatorial chemistry & high throughput screening 15(7):542-550

Zheng Y, Liu T, Wang Z, Xu Y, Zhang Q, Luo D (2018) Low molecular weight fucoidan attenuates liver injury via SIRT1/AMPK/PGC1α axis in db/db mice. International Journal of Biological Macromolecules 112:929-936

Zhou J, Xu G, Yan J, et al. (2015a) Rehmannia glutinosa (Gaertn.) DC. polysaccharide ameliorates hyperglycemia, hyperlipemia and vascular inflammation in streptozotocin-induced diabetic mice. Journal of ethnopharmacology 164:229-238

Zhou J, Yan J, Bai Z, Li K, Huang K (2015b) Hypoglycemic activity and potential mechanism of a polysaccharide from the loach in streptozotocin-induced diabetic mice. Carbohydrate polymers 121:199-206

Zhou Q-L, Wang Z, Chen W-T, et al. (2024) The structural characteristics, biological activities and mechanisms of bioactive brown seaweed polysaccharides: A review. Journal of Functional Foods 119:106303

Zhu J, Liu W, Yu J, et al. (2013a) Characterization and hypoglycemic effect of a polysaccharide extracted from the fruit of Lycium barbarum L. Carbohydrate polymers 98(1):8-16

Zhu K, Nie S, Gong D, Xie M (2016) Effect of polysaccharide from Ganoderma atrum on the serum metabolites of type 2 diabetic rats. Food Hydrocolloids 53:31-36

Zhu K, Nie S, Li C, et al. (2013b) A newly identified polysaccharide from Ganoderma atrum attenuates hyperglycemia and hyperlipidemia. International journal of biological macromolecules 57:142-150

Zhu K-X, Nie S-P, Li C, Gong D, Xie M-Y (2014) Ganoderma atrum polysaccharide improves aortic relaxation in diabetic rats via PI3K/Akt pathway. Carbohydrate polymers 103:520-527 Zhu Z, Zhou Q, Sun D-W (2019) Measuring and controlling ice crystallization in frozen foods: A review of recent developments. Trends in Food Science & Technology 90:13-25

Zorena K, Michalska M, Kurpas M, Jaskulak M, Murawska A, Rostami S (2022) Environmental factors and the risk of developing type 1 diabetes—old disease and new data. Biology 11(4):608

Zou M-Y, Nie S-P, Yin J-Y, Xie M-Y (2020) Ascorbic acid induced degradation of polysaccharide from natural products: a review. International Journal of Biological Macromolecules 151:483-491

CHAPTER 2
MATERIALS AND METHODS

2.1 Collection of Seaweeds

Ecklonia maxima and *Gracilaria gracilis* were collected from Kelpak in Cape Town, and Wild Coast Abalone, East London, South Africa, respectively. They were identified and deposited at the herbarium of the Department of Botany of Nelson Mandela University, South Africa (32°45.048'S. 28°16.558′E), and assigned the voucher codes, D1 and D2.

Salt and particles were washed from both seaweeds by rinsing thoroughly with distilled water. The seaweeds were air-dried before subjecting to freeze-drying. The dried samples were blended to fine powder, from which 200 g was defatted with n-hexane by soaking in 1000 mL of the latter in a flat bottom flask at room temperature for 2 days with constant shaking. The solvent was decanted, and the defatted samples were dried overnight in a fume cupboard.

2.2 Extraction of Sulphated Polysaccharides

Sulphated polysaccharides (SPs) were extracted from the seaweed samples using the cold and hot water extraction methods as previously described (Olasehinde et al. 2020; Pengzhan et al. 2003). For the hot water extraction, 100 g of the defatted samples was mixed with 500 ml of distilled water and autoclaved for 15 min. A Büchner funnel lined with a Whatman TG 100 Separating Gauze was used in filtering the slurry. The filtrates were collected and precipitated overnight with ethanol and then centrifuged at 1800 rpm for 10 min at 25 °C. The resulting pellets were lyophilized, blended and stored at 4 °C until further use.

2.3 Shelf Life/Stability Study

The extracted SPs were subjected to shelf-life study to determine their stability and biological activities at different storage conditions (Bajaj et al. 2017). Freshly prepared SPs (30 mg) were

stored in sterile 50 mL falcon tubes at different storage conditions consisting of different ranges of temperatures and time as shown in Table 2.1.

Table 2.1: Shelf life study design.

Storage Temperature (°C)	Sampling Time Points (Months)	Container
25	0, 1, 2, 3, 4, 5 and 6	Closed
37	0, 1, 2, 3, 4, 5 and 6	Closed
4	0, 1, 2, 3, 4, 5 and 6	Closed

At every sampling time point, SPs were assayed for total sulphate and sugar contents, as well as antidiabetic activities *in vitro* via their inhibitory effect on α-amylase and α-glucosidase activities

2.3.1 Determination of Total Sulphate Content

The total sulphate content of the SPs was determined using the $BaCl_2$-gelatin turbidity method (Bhadja et al. 2016). Briefly, 0.3 % gelatine solution was prepared in hot water (60–70 °C) and stored at 4 °C overnight. $BaCl_2$-gelatin reagent was prepared by dissolving 2 g of $BaCl_2$ in gelatin solution and allowed to stand for 2–3 h at 25 °C. SPs (0.2 mL) dissolved in distilled water (1 mg/mL) were mixed with 3.8 ml of 0.5 M HCl and 1 ml of $BaCl_2$-gelatin reagent. The mixture was allowed to stand for 20 min. Absorbance was read at 360 nm using a microplate reader (SpectraMax M2, Molecular Devices, San Jose, CA, USA). The total sulphate was extrapolated from a standard graph of potassium sulphate (0 – 100 μg/mL).

2.3.2 Total Sugar content

The total sugar content of the SPs was determined using the phenol–sulfuric acid method (DuBois et al. 1956). Stock solution (1 mg/mL) of the SPs were prepared with distilled water, from which a series of dilutions consisting of 20, 40, 80, 120, 160, and 200 μg/mL were further prepared. 1 mL of 5 % phenol solution and 5 mL of H_2SO_4 were added to each dilution and allowed to incubate for 10 min at room temperature. The reaction mixtures were vortexed and heated in a water bath

at 25-30 ℃ for 30 min. Absorbance was read at 490 nm using a microplate reader (SpectraMax M2, Molecular Devices, San Jose, CA, USA). Total glucose was extrapolated from a standard graph of glucose (prepared with same procedure above).

2.3.3 *in vitro* Antidiabetic Activities

The SPs were analysed for their *in vitro* antidiabetic activities by investigating their abilities to inhibit the activities of α-glucosidase and α-amylase, using previously described methods (Oboh and Ademosun 2011).

2.3.3.1 α-glucosidase Inhibition

About 50 µL of each SP concentration or acarbose (standard antidiabetic drug) were added to an equal volume of α-glucosidase (1.0 U/mL) in 100 mM phosphate buffer (pH 6.8) in a 96-well plate and incubated for 15 min at 37°C. 100 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) solution in 100 mM phosphate buffer (pH 6.8) was then added to the reaction mixture, and further incubated at 37 °C for 20 min. Absorbance was read at 405 nm. The percentage inhibitory activity was calculated using the formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(Absorbance\ of\ control)}\ x\ 100$$

Where control consists of a reaction mixture without inhibitors.

2.3.3.2 α-amylase inhibition

About 50 µL of each SP concentration or acarbose was mixed with equal volumes of porcine pancreatic amylase (2 U/ml) in 100 mM phosphate buffer (pH 6.8) and incubated for 10 min. 50 µL of 1 % starch solution in 100 mM phosphate buffer (pH 6.8) was added to the reaction mixture and further incubated for 10 min. 100 µL of the dinitrosalicylate (DNS) reagent was then added to the reaction mixture and boiled for 10 min. Absorbance was read at 540 nm.

The percentage inhibitory activity of the SPs was calculated using the following formula:

Where control consists of a reaction mixture without inhibitors.

$$\% \text{ Inhibition} = \frac{(Absorbance\ of\ Control - Absorbance\ of\ Sample)}{(Absorbance\ of\ COntrol)} \times 100$$

2.4 Animals

Fifty-one (51) male Sprague Dawley (SD) rats weighing 180 – 200 g were obtained from the Biomedical Research Unit (BRU), University of KwaZulu-Natal, Durban, South Africa. They were acclimatized on pelletized chows and water (*ad libitum*) for 7 days, and subjected to natural photo period of 12-h light-dark cycle. The study was carried out under the approval of the Animal Ethics Committee, University of KwaZulu-Natal, Durban, South Africa (Ethical Approval Number: AREC/00002347/2021).

2.5 Animal Groupings

After acclimatization, the animals were randomly grouped as follows:

Negative Control (NC): Normal rats (non-diabetic)

Positive Control (PC): untreated diabetic rats

Diabetic + low dose (DBL): diabetic rats administered low dose (150 mg/kg bodyweight [bw]) of *E. maxima*

Diabetic + high dose (DBH): diabetic rats administered high dose (300 mg/kg bw) of *E. maxima*

Diabetic + standard drug metformin (DBM): diabetic rats administered 200 mg/kg bw of metformin

Negative Toxicological Control (NTB): Non-diabetic rats administered high dose of *E. maxima*

Diabetic + low dose (DRL): diabetic rats administered low dose (150 mg/kg bw) of *G. gracilis*

Diabetic + high dose (DRH): diabetic rats administered high dose (300 mg/kg bw) of *G. gracilis*

Negative Toxicological Control (NTR): Non-diabetic rats administered high dose of *G. gracilis*.

The negative groups (NC, NTR and NTB) consisted of 5 rats each, while the others consisted of 6 rats each.

2.6 Induction of type 2 diabetes

Following adaptation, T2D was induced in the diabetic groups using the fructose- streptozotocin protocol (Wilson and Islam, 2012). Briefly, 10% fructose was provided *ad libitum* to the diabetic groups (DC, DBL, DBH, DBM, DRL, and DRH) for 2 weeks. After overnight fasting, the rats were injected intraperitoneally with streptozotocin (40 mg/kg bw) in citrate buffer (pH 4.5). The normal groups (NC, NTB and NTR) were injected intraperitoneally with citrate buffer only. After 7 days, non-fasting blood glucose levels were measured with a glucometer (Accu-Chek®), and rats with blood glucose level was >200 mg/dL, were considered diabetic.

2.7 Intervention trial

Following induction of T2D, DBL, DBH, and NTB were orally administered *E. maxima* SPs; while *G. gracilis* SPs were orally administered to DRL, DRH, and NTR groups. Distilled water was administered to NC and PC groups, while metformin was administered to DBM. The rats were treated for 5 times a week for an intervention period of 4 weeks. Blood glucose level and body weight were monitored weekly and provided in Appendix 1.

2.8 Sacrifice and Collection of Liver Tissues

The rats were humanely sacrificed by euthanizing with isoflurane after an overnight (8 h) fasting. Blood was collected via cardiac puncture into sterile plain tubes. The rats were dissected and their livers were harvested. The organs were rinsed off blood stains with normal saline, mopped with filter paper and weighed. About 0.5 g of each liver was excised and homogenized in 5 mL of 50 mM sodium phosphate buffer (pH 7.5) containing 10 % triton X-100. The homogenized samples

were centrifuged at 15,000 rpm for 10 min at 4 °C. The supernatants were collected into 2 mL Eppendorf tubes and stored at -80°C for further analysis.

2.9 Glucogenic Enzymes Activities

The liver supernatants were investigated for activities of glucogenic enzymes such as fructose1,6-biphosphatase, glucose 6-phosphatase and glycogen phosphorylase using previously described protocols (Balogun and Ashafa 2017; Cornblath et al. 1963; Erukainure et al. 2017; Gancedo and Gancedo 1971; Mahato et al. 2011).

2.9.1 Fructose-1,6-Bisphosphatase Activity

100 µL of the tissue supernatant was added to 100 µL of 0.05 M fructose, 1200 µL of 0.1 M Tris–HCl buffer (pH 7.0), 250 µL 0.1 M $MgCl_2$, 100 µL 0.1 M KCl, and 250 µL 1 mM EDTA at 37°C and incubated for 15 min. The reaction was stopped with 10% TCA and centrifuged at 3000 rpm (4 °C) for 10 mins. 50 µL of freshly prepared 9% ascorbic acid and 1.25% ammonium molybdate were added to 100 µL of the resulting supernatant in a 96-well plate. The reaction mixture was allowed to stand for 20 mins at room temperature, and absorbance was measured at 680 nm. The enzyme activity was extrapolated from an inorganic phosphate (Pi) standard graph.

2.9.2 Glucose-6 phosphatase activity

200 µL of the tissue supernatant was added to 100 µL of 0.25 M glucose 6 phosphatase, 200 µL of 5 mM KCl, 1300 µL of 0.1 M Tris-HCl buffer and incubated in a shaker for 30 min at 37 °C. The reaction was stopped with 1 mL of distilled water and 1.25 % ammonium molybdate. 1 mL of freshly prepared 9% ascorbate was then added to the reaction mixture and allowed to stand for 30 min. Absorbance was measured at 660 nm. The enzyme activity was extrapolated from an inorganic phosphate (Pi) standard graph.

2.9.3 Glycogen phosphorylase activity

100 µL of the tissue supernatant was added to 64 mM glucose-1-phosphate and 4 % glycogen and incubated for 10 min at 30 °C. The reaction was stopped with 20 % ammonium molybdate in concentrated H_2SO_4. The reaction mixture was further incubated with Elon reducer and distilled water at 30 °C for 45 min. Absorbance was read at 340 nm. The enzyme activity was extrapolated from an inorganic phosphate (Pi) standard graph. The enzyme activity was extrapolated from an inorganic phosphate (Pi) standard graph.

2.10 Evaluation of antioxidant and oxidative stress markers

The liver tissues were assayed for oxidative stress biomarkers including reduced glutathione (GSH) level, superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase, glutathione-S-transferase, catalase activities, and malondialdehyde (MDA) level, according to previous methods (Aebi 1984; Bauman et al. 1988; Chowdhury and Soulsby 2002;

Ellman 1959; Habig et al. 1974; Janero 1990; Jelodar et al. 2020).

2.10.1 Reduced Glutathione Level

About 150 µL of the tissue supernatant was mixed with an equal volume of 10% TCA and centrifuged at 2000 rpm for 10 min at room temperature. 80 µL of the supernatant was mixed with 40 µL of 0.5 mM DTNB in a 96-well plate. The reaction mixture was further incubated with 200 µL of 0.2M phosphate buffer (pH 7.8) for 15 min at room temperature. Absorbance was read at 415 nm. The GSH level was extrapolated from a GSH standard graph.

2.10.2 Glutathione Reductase Activity

About 10 µL of the tissue supernatant was mixed with 221 µL of 50 mM Tris-HCl buffer (containing 1 mM EDTA, pH 8.0) and 38 µL of 8 mM oxidized glutathione (GSSG) in a 96-well plate. 10 µL of NADPH was thereafter added to the reaction mixture. Absorbance was read at 340

nm at a 2 min interval for 8 min using a microplate reader. The activity was calculated using this expression:

$$\text{Activity} = 1000\ X\ [\frac{A1-Ab}{\quad}]\ X\ 0.5\ \mu\text{mol/min/}\mu\text{g of protein}\ \varepsilon_{340}$$

Where: ε_{340} is the molar absorptivity at 340 nm which is 6.22 mM/cm, A1 and Ab represents rate of the sample and blank reactions.

2.10.3 Glutathione peroxidase activity

About 5 µL of the tissue supernatant was added to 210 µL of phosphate buffer (pH 6.9), 2.5 µL of 100 mM GSH, and 5 µL of distilled H_2O in a 96-well plate, and incubated for 10 min at 37 ºC. 2.5 µL of 15 mM NADPH was thereafter added to the reaction mixture, followed by 10 µL of 1 mM DTNB. Absorbance was at 412 nm using a microplate reader. A GSH standard graph was used to extrapolate the glutathione peroxidase activity.

2.10.4 Superoxide Dismutase Activity

A 170 µL of 0.1 mM diethylenetriaminepentaacetic acid (DETAPAC) was added to and 15 µL of the tissue supernatants in a 96-well plate. 15 µL of 1.6 mM 6-HD was then added and mixed by gently tapping all four sides of plates. Absorbance of the resulting mixture was measured at 492 nm for 5 mins at 1 min interval. The enzyme activity was calculated by the formula:

$$\text{Activity} = 1000 \text{ x } [(A_1\text{-}A_b)/\varepsilon 490] \text{ x } 0.5 \text{ nmol/min/}\mu\text{g protein}$$

$\varepsilon 490$ = Molar absorptivity at 490nm = 1.742/mM/cm

A_1 and A_b = Reaction rate for sample and blank respectively

2.10.5 Catalase activity

10 µL of the tissue supernatant was mixed with 340 µL of 50 mM sodium phosphate buffer (pH 7.0). 150 µL of 2 M H_2O_2 was added to the reaction mixture. Absorbance was read at 240 nm for 3 min at 1 min interval.

2.10.6 Glutathione-S-Transferase Activity

A reaction mixture of 0.1 mL of 0.25 M phosphate buffer (pH 6.5), 25 mM 1- chloro-2,4dinitrobenzene (CDNB) and 0.7 mL of distilled water was incubated for 5 mins at 37ºC. 25 µL of the tissue supernatants and 20 µL of 25 mM glutathione were added to the reaction mixture. Absorbance was measured at 340 nm at 5 min interval. Blank consisted of reaction mixture without enzyme.

2.10.7 Lipid Peroxidation Level

Lipid peroxidation levels of the tissues were determined by measuring thiobarbituric acid reactive substances (TBARS), expressed as malondialdehyde (MDA) equivalent. 100 µL of the samples were mixed with an equal volume of 8.1% SDS solution, 375 µL of 20% acetic acid, 1 mL of 0.25% thiobarbituric acid (TBA), and 425 µL of distilled water. The reaction mixture was boiled for 1 h in a water bath. Thereafter, 200 µL of the heated mixture was collected into 96-well plate and absorbance read at 532 nm. TBARS concentration was extrapolated from a MDA standard graph.

2.11 Acetylcholinesterase Activity

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

2.12 Hepatic Function Markers

The collected blood samples were centrifuged at 10,000 rpm at 4◦C for 10 min. The supernatant (serum) was collected into 2 mL Eppendorf tubes and analyzed for serum hepatic function markers

which includes for aspartate transaminase (AST) and alanine transaminase (ALT), using an Automated Chemistry Analyzer (Labmax Plenno, Labtest Inc., Costa Brava, Brazil).

2.13 Metabolic Profiling

About 0.5 g of the liver was excised and homogenized in cold methanol/ethanol (2:8) to extract the lipid metabolites (Erukainure et al. 2020). The homogenized tissues were incubated on ice for 20 min (Chan et al. 2013), and thereafter, centrifuged at 15,000 rpm at 4 °C for 10 min. The supernatants were decanted into glass vials for subsequent analysis.

2.14 Metabolic Profiling with Gas Chromatography-Mass Spectrometry (GC-MS)

The extracted metabolites were profiled with GC-MS using an Agilent technologies 6890 Series GC coupled with (an Agilent) 5973 Mass Selective detector and driven by Agilent chemstation software. The metabolites were separated with a HP-5MS capillary columns. 1 µL of the tissue supernatants was injected in a splitless mode. Carrier gas: ultra-pure helium used as the carrier gas; Flow rate: 60 mL h^{-1}; Initial oven temperature: 60 °C for 2 min; Final oven temperature: 285 °C; Hold time: 3 min; Ion source and quadrupole Temperatures: 230 °C, respectively; Electron ionization mode and electron multiplier voltage: 70 eV and 1859 V, respectively. The metabolites were identified by direct mass spectral comparison using an inbuilt

NIST mass spectral library.

2.15 Pathway analysis

The identified metabolites were subjected to pathway enrichment to identify the most relevant pathways involved in lipid metabolism in diabetic hepatopathy in T2D, using the MetaboAnalyst 6.0 online server (https://www.metaboanalyst.ca/) (Pang et al. 2024).

2.16 Statistical Analysis

Clustering analysis of the GC-MS identified metabolites which covers for heat maps and principal component analysis (PCA), was carried out with MetaboAnalyst 6.0 online server (https://www.metaboanalyst.ca/) (Pang et al. 2024).

CHAPTER 3

Sulphated Polysaccharides from *Ecklonia maxima* (Brown Seaweed) from Papenfuss Protects against Diabetic Hepatopathy by Mitigating Glucolipotoxicity and Oxidative Stress in Hepatic Tissues of Type- 2

[1]Lethiwe B. Mpungose, [1]Tosin A. Olasehinde, [1,2]Ochuko L. Erukainure, [2]Md. Shahidul Islam, [1]Ademola O. Olaniran*

1. Department of Microbiology, School of Life Sciences, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa.
2. Department of Biochemistry, School of Life Sciences, University of Kwazulu-Natal, Westville Campus, Durban 4000, South Africa.

***Correspondence:** olanirana@ukzn.ac.za

Abstract

Diabetic hepatopathy is among the major contributor to mortality and morbidities associated with type 2 diabetes (T2D). Sulphated polysaccharides (SPs) from seaweeds have been reported for their protective effects in T2D and its complications. The present study investigated the protective effect of SPs from *Ecklonia maxima* on diabetic hepatopathy in hepatic tissues of T2D rats. Two groups of T2D rats were administered 150 and 300 mg/Kg bodyweight (bw) of SPs, respectively. Water was administered to the T2D (positive control) and negative control groups, while metformin served as the standard antidiabetic drug. Normal rats administered 300 mg/Kg bw SPs served as the toxicology group. Induction of T2D significantly depleted glutathione level, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase, while concomitantly increasing malondialdehyde level, fructose-1.6-biphosphatase, glucose 6-phosphatase, glycogen phosphorylase, and acetylcholinesterase activities in hepatic tissues. Furthermore, T2D dysregulated glucose, lipid and amino acid metabolic pathways, and their metabolites. Treatment with SPs significantly reversed the glutathione and malondialdehyde levels, and enzymes activities, while concomitantly upregulating the studied metabolic pathways and their metabolites.

Thus, indicating that treatment with SPs from *E. maxima* protects against diabetic hepatopathy in T2D by improving glucose and amino acid metabolisms, while concomitantly attenuating lipotoxicity, oxidative stress and inflammation.

Keywords: Glucotoxicity; Hepatopathy; Oxidative stress; Brown seaweeds; Sulphated polysaccharides; and Type 2 Diabetes.

3.1 Introduction

Type 2 diabetes (T2D) is ranked as the most prominent of all diabetes types as it contributes to over 90% of all diabetes-related morbidities and mortalities (IDF 2025). It is characterized by insulin resistance and pancreatic β-cells dysfunction, leading to hyperglycemia. An increase in hyperglycemia has been linked to the continuous generation of reactive oxygen species (ROS) and free radicals, which overwhelm the body's oxidant-antioxidant balance in favor of the former. This imbalance leads to oxidative stress, which has been implicated in the pathogenesis and progression of T2D and its complications (Diyorbek 2025; Ren et al. 2025). Among these complications is diabetic hepatopathy.

Diabetic hepatopathy is a spectrum of liver diseases including non-alcoholic fatty liver disease (NAFLD), hepatocellular carcinoma, abnormal liver enzymes, cirrhosis, and acute liver failure (Islam et al. 2020; Mobasheri et al. 2023; Tolman et al. 2007). Insulin resistance arising from T2D, alters the uptake of glucose and its utilization in the hepatic tissues, thereby increasing its output by the liver (Erukainure et al. 2021). Alterations in lipid metabolic pathways and their metabolites have also been implicated in diabetic hepatopathy (Erukainure et al. 2021; Hazlehurst et al. 2016). It is attributed to increased hepatic fatty acids uptake, *de novo* lipogenesis, suppressed secretion of very low-density lipoprotein (VLDL), leading to hypertriglyceridemia and hypercholesteremia (Mobasheri et al. 2023; Westcott et al. 2024). Furthermore, dysmetabolism of amino acids also exacerbates hepatic production of glucose, which increases the tissues insensitivity to insulin leading to glucotoxicity (Ding et al. 2023; Zhu et al. 2022). An interplay of these alterations is considered glucolipotoxicity, which exacerbates the production of ROS and free radicals, leading

to oxidative stress (Ma et al. 2021). Further contributing to the pathophysiology of diabetic hepatopathy.

The medicinal properties of sulphated polysaccharides (SPs) from seaweeds have been explored in the treatment of various diseases including T2D and its complications (Jia et al. 2020; Zhong et al. 2021). Among these SPs, are those extracted from *Ecklonia maxima*, which is endemic to South Africa (Pillay et al. 2025). The antidiabetic properties of *E. maxima* SPs have been attributed to its ability to inhibit glucose metabolizing enzymes activities and intestinal glucose absorption, while stimulating muscle glucose uptake (Daub et al. 2020; Pillay et al. 2025). The SPs have also been reported for its potent antioxidant and antiinflammatory activities (Lee et al. 2021; Nagahawatta et al. 2022), as well as its neuroprotective effect (Olasehinde et al. 2019a).

However, *E. maxima* SPs have not been fully explored for its protective potential in diabetic hepatopathy. Thus, the present study was aimed at exploring the protective potentials of SPs extracted from *E. maxima* on diabetic hepatopathy, by investigating its effect on glucose, lipid and amino acid metabolisms, oxidative stress and cholinesterase activity in hepatic tissues of T2D rats.

3.2 Materials and Methods

Kindly refer to sections 2.1, 2.2, and 2.4 – 2.15 in Chapter 2.

3.3 Results

As shown in Figures 3.1A – C, induction of T2D led to significant ($p<0.05$) increase in the hepatic activities of fructose-1,6-biphosphatase, glucose 6-phosphatase and glycogen phosphorylase. These activities were significantly ($p<0.05$) reversed following treatment with SPs and compared favorably with the normal (NC) and metformin-treated (DBM) groups.

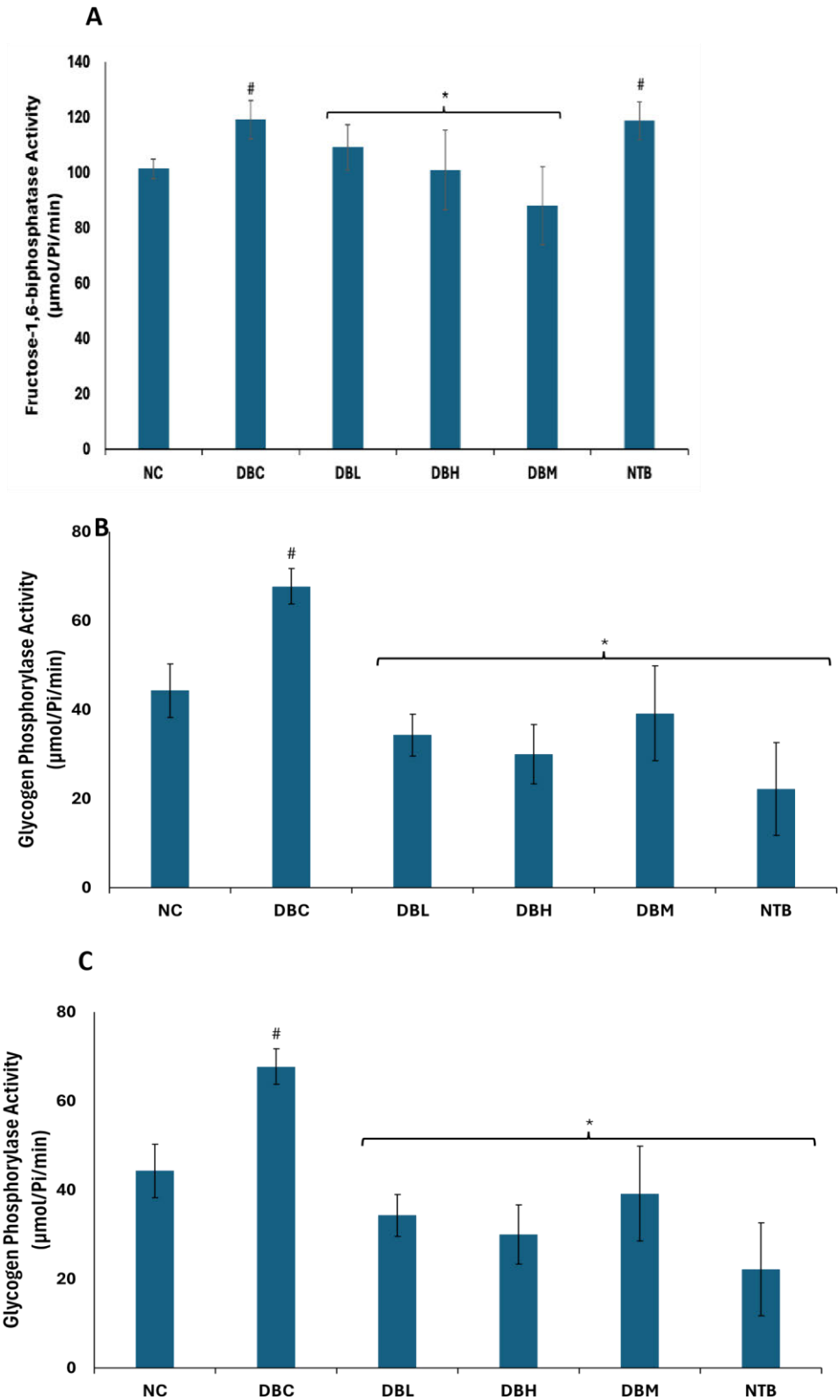
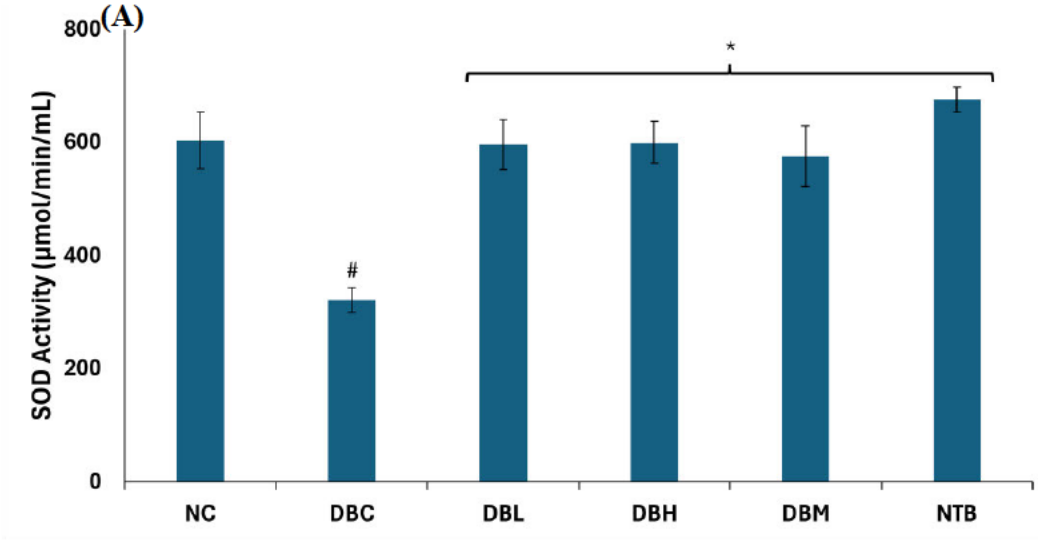


Figure 3.1: Effect of Sulphated Polysaccharides on (A) fructose-1,6-biphosphatase, (B) glucose 6-phosphatase, and (C) glycogen phosphorylase activities in hepatic tissues of Type-2 Diabetic rats. Values = mean±SD; n=5.

*Statistically significant ($p<0.05$) to DBC; #statistically ($p<0.05$) significant to NC).

The hepatic SOD and catalase activities were significantly ($p<0.05$) depleted following induction of T2D, as shown in Figures 3.2A and 3.2B. Treatment with both doses of SPs significantly ($p<0.05$) elevated the enzymes activities and compared favorably with NC and DBM.



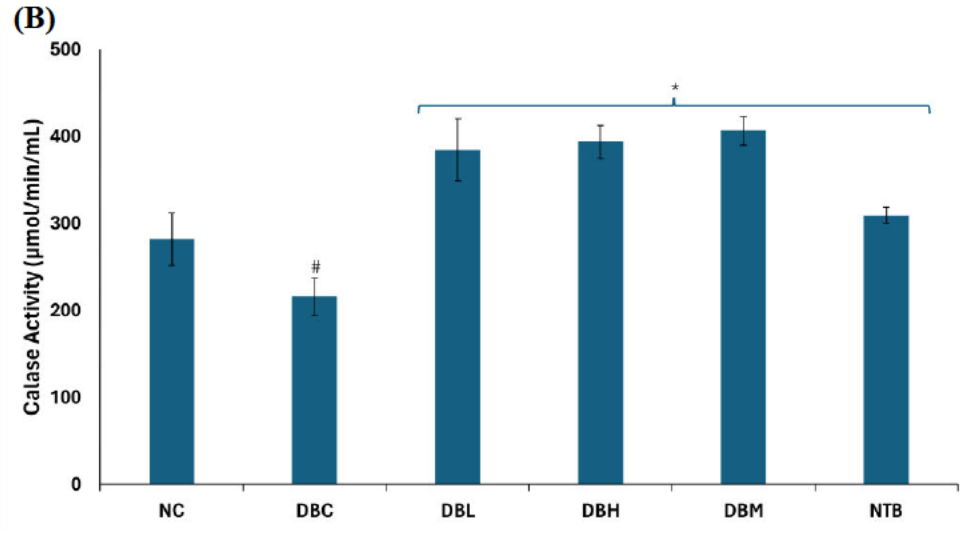


Figure 3.2: Effect of Sulphated Polysaccharides on (A) Superoxide Dismutase (SOD), and (B) catalase activities in hepatic tissues of Type-2 Diabetic rats. Values = mean±SD; n=6. *Statistically significant ($p<0.05$) to DBC; #statistically ($p<0.05$) significant to NC).

Induction of T2D significantly ($p<0.05$) elevated GSH level, with concomitant elevated activities of glutathione reductase, glutathione peroxidase and glutathione S- transferase as shown in Figures 3.3A – D. These protein level and enzymes activities were significantly ($p<0.05$) elevated following treatment with SPs. However, administration of SPs at high dose to normal rats (NTB), did not elevate the activities of glutathione reductase and glutathione S- transferase.

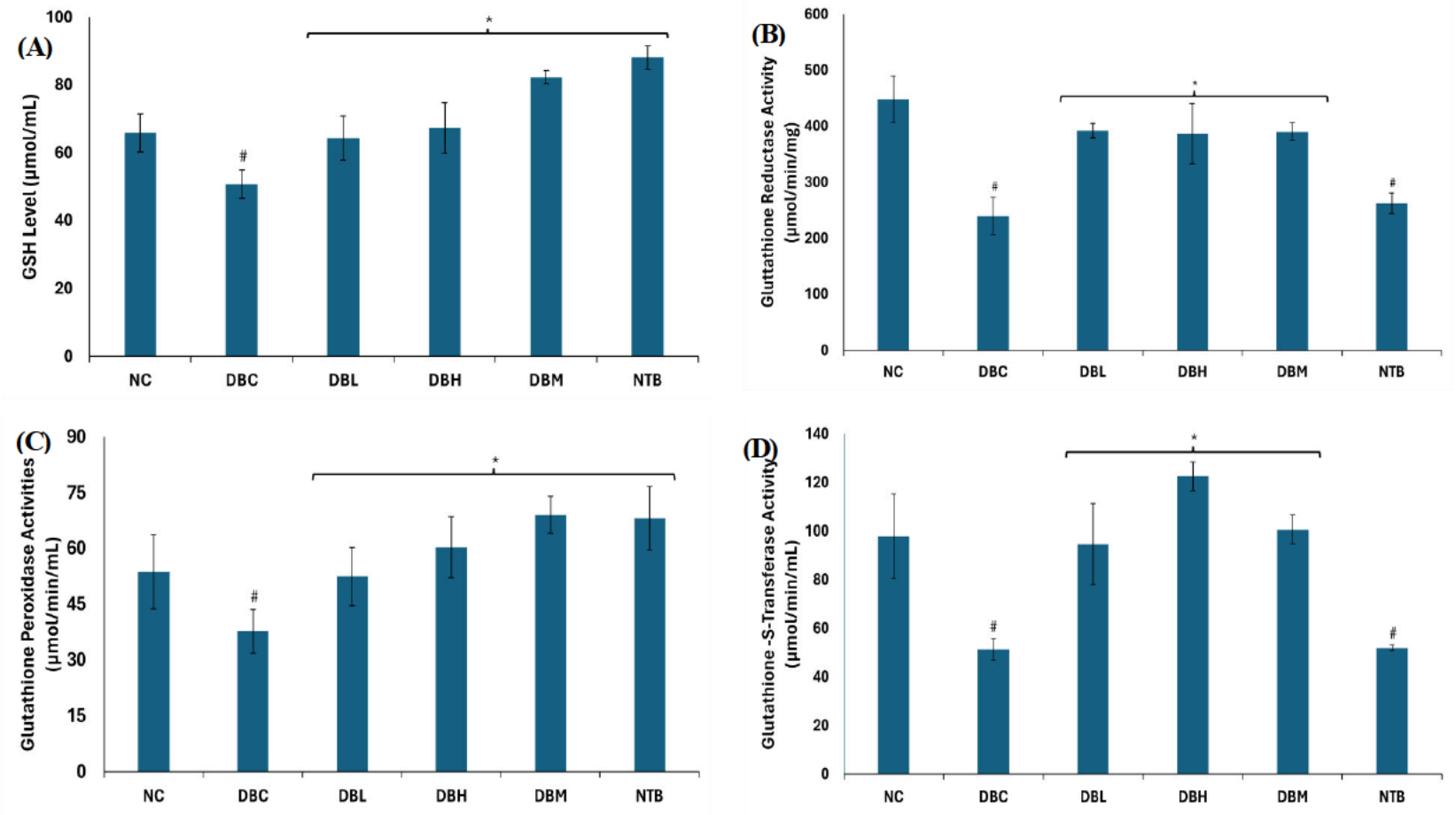


Figure 3.3: Effect of Sulphated Polysaccharides on (A) Glutathione (GSH) level, (B) glutathione reductase, (C) glutathione peroxidase, and (D) glutathione-s-transferase activities in hepatic tissues of Type-2 Diabetic rats. Values = mean±SD; n=6. *Statistically significant ($p<0.05$) to DBC; #statistically ($p<0.05$) significant to NC).

As shown in Figure 3.4, induction of T2D significantly ($p<0.05$) elevated the hepatic MDA level. The level was significantly ($p<0.05$) depleted following treatment with SPs.

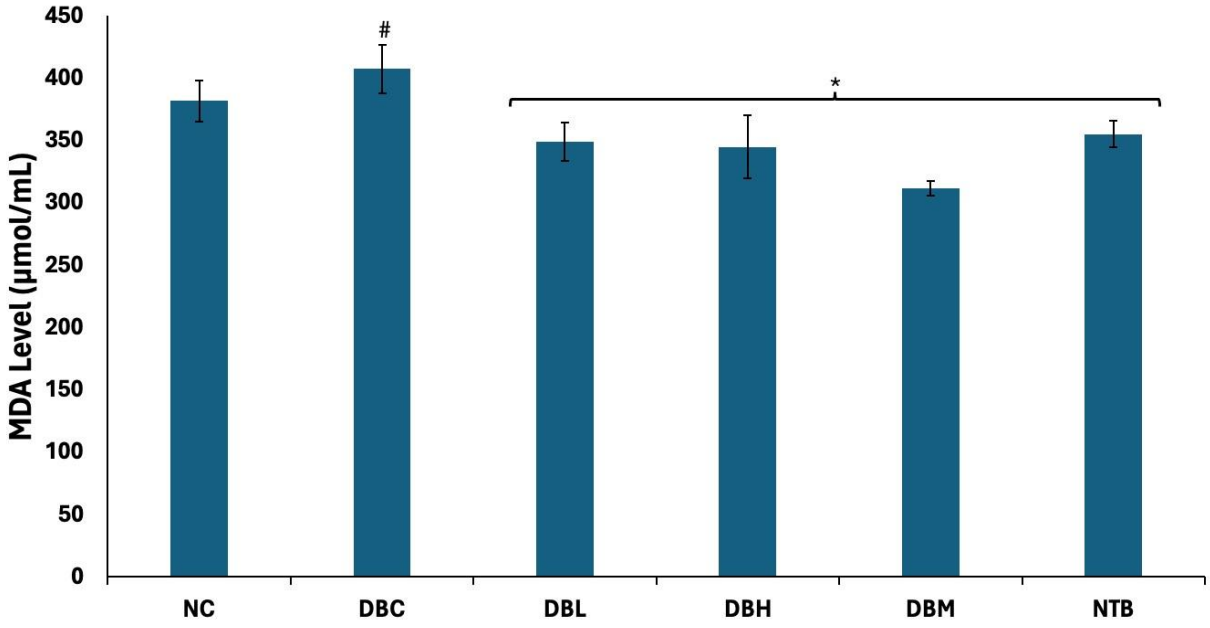


Figure 3.4: Effect of Sulphated Polysaccharides on Mass Drug Administration (MDA) level in hepatic tissues of Type-2 Diabetic rats. Values = mean±SD; n=6.

*Statistically significant ($p<0.05$) to DBC; #statistically ($p<0.05$) significant to NC).

As shown in Figure 3.5, hepatic activity of acetylcholinesterase was significantly ($p<0.05$) elevated on induction of T2D. The activity was significantly ($p<0.05$) suppressed following treatment with SPs, and compared favorably with NC and DBM.

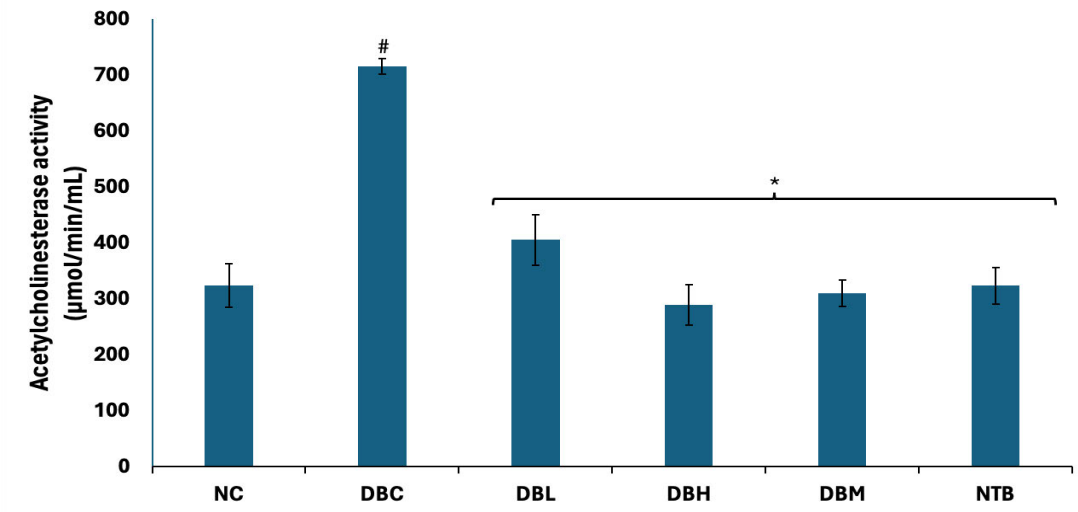


Figure 3.5: Effect of SPs on acetylcholinesterase activity in hepatic tissues of Type-2 Diabetic rats. Values = mean±SD; n=6. *Statistically significant ($p<0.05$) to DBC; #statistically ($p<0.05$) significant to NC).

As shown in Table 3.1, GC-MS analysis of the hepatic tissues of normal rats revealed the presence of urea, alpha-ketoglutaric acid, palmitoleic acid, pentadecanoic acid, linoleic acid, (9e)-9octadecenoic acid, cis-vaccenic acid, octadecanoic acid, arachidonic acid, 12-ketotridecanoic acid eicosapentaenoic acid, cholesteryl nonanoate, cholesterol and dihydrobrassicasterol. These metabolites were altered following induction of T2D, with depletion in cis-vaccenic acid, arachidonic acid, eicosapentaenoic acid and dihydrobrassicasterol, while concomitantly generating 2-hexyloctanoic acid, nicotinamide, heptadecanoic acid, linolenic acid, docosahexanoic acid, squalene, cholestadiene and 24-dehydrocholesterol. Treatment with SPs at low dose led to the regeneration of arachidonic acid, with concomitant generation of mesaconic acid, isobarbituric

acid, furyl hydroxymethyl ketone, tetradecanoic acid, 17α-hydroxypregnenolone, eicosanoic acid, cholesterol benzoate and deaminoisocytosine. It depleted T2D-generated metabolites except 2hexyloctanoic acid and heptadecanoic acid. At high dose, treatment with the SPs led to the depletion of T2D-generated metabolites except 2-hexyloctanoic acid and squalene, while concomitantly generating 4,5-diamino-2-pyrimidinol. Treatment with metformin also led to the depletion of T2D-generated metabolites except cholestadiene, and concomitantly generated eicosanoic acid, deaminoisocytosine and 4,5-diamino-2-pyrimidinol.

Table 3.1: Effect of Sulphated Polysaccharides on hepatic metabolites of Type-2 Diabetic rats.

Metabolites	NC	DBC	DBL	DBH	DBM	NTB
Urea	0.47□0.34	0.62□0.29	ND	ND	ND	ND
alpha-Ketoglutaric acid	0.65□0.45	1.40□0.79	1.25□0.88	1.13□0.57	1.01□0.64	1.21□0.53
Palmitoleic acid	1.38□0.17	1.53□0.25	0.50□0.12	1.57□0.46	1.82□0.68	1.91□0.60
Pentadecanoic acid	12.14□4.12	8.02□1.81	0.38□0.21	7.92□2.21	8.18□1.02	8.28□2.19
Linoleic acid	1.53□0.19	3.91□1.08	0.53□0.08	1.30□0.41	5.19□1.14	1.30□0.41
(9E)-9-Octadecenoic acid	6.44□0.30	3.43□0.41	12.12□5.92	ND	3.90□0.38	3.79□0.25
cis-Vaccenic acid	3.99□1.27	ND	ND	ND	ND	ND
Octadecanoic acid	4.61□0.05	4.21□0.37	12.62□3.53	4.27□0.54	5.60□2.41	5.76□1.32
Arachidonic acid	0.87□0.38	ND	5.68□0.51	4.41□0.56	4.01□0.07	ND
12-Ketotridecanoic acid	0.68□0.08	0.75□0.12	ND	ND	ND	ND
Eicosapentaenoic acid	0.98□0.08	ND	0.99□0.08	0.82□0.09	ND	ND
Cholesteryl nonanoate	0.74□0.19	0.28□0.10	0.68□0.09	ND	ND	ND
Cholesterol	18.99□4.38	23.99□6.06	17.60□2.20	19.38□5.93	20.80□4.05	21.37□6.61
Dihydrobrassicasterol	2.04□0.36	ND	ND	ND	ND	ND
2-Hexyloctanoic acid	ND	2.99□0.18	2.84□0.33	0.68□0.38	ND	2.50□0.12
Nicotinamide	ND	0.51□0.08	ND	ND	ND	ND
Heptadecanoic acid	ND	0.29□0.12	0.52□0.19	ND	ND	ND
Linolenic acid	ND	0.57□0.10	ND	ND	ND	ND
Docosahexanoic acid	ND	0.66□0.08	ND	ND	ND	ND
Squalene	ND	3.19□1.25	ND	1.32□0.58	ND	1.89□0.87
Cholestadiene	ND	0.88□0.38	ND	ND	0.96□0.02	0.54□0.22

24-Dehydrocholesterol	ND	0.23□0.01	ND	ND	ND	ND
Mesaconic acid	ND	ND	0.40□0.23	ND	ND	ND
Isobarbituric acid	ND	ND	0.45□0.25	ND	ND	ND
Furyl hydroxymethyl ketone	ND	ND	0.38□0.08	ND	ND	ND
Tetradecanoic acid	ND	ND	0.37□0.19	ND	ND	ND
17α-Hydroxypregnenolone	ND	ND	0.22□0.13	ND	ND	ND
Eicosanoic acid	ND	ND	5.46□0.14	ND	5.30□0.36	4.80□0.65
Cholesterol benzoate	ND	ND	0.20□0.07	ND	ND	ND
Deaminoisocytosine	ND	ND	0.71□0.44	ND	0.64□0.40	0.58□0.29
Cholesteroyl-oleate	ND	ND	ND	0.42□0.20	ND	ND
4,5-Diamino-2-pyrimidinol	ND	ND	ND	ND	0.48□0.30	ND
4,5-Diamino-6hydroxypyrimidine	ND	ND	ND	ND	ND	0.14□0.07

Values = mean±SD; n=6. ND = not detected.

As shown in Figure 3.6A, heatmap analysis revealed the distinct distribution of the hepatic metabolites among the treatment groups, with the DBL and DBC group portraying the most distinct changes as depicted by the positive value and color intensity. This was further portrayed by principal cluster analysis (PCA), which also depicted distinct changes in hepatic metabolites distribution between the SPs treated groups and untreated diabetic group as shown by the score plots (Figure 3.6B). Biplot analysis of PCA components, PC1 (32.42%) and PC2 (29.48%) revealed strong relationships between treatment groups and the hepatic metabolites: urea, squalene, pentadecanoic acid, 4,5-diamino-2-pyrimidinol, and eicosapentaenoic acid, as shown in Figure 3.6C.

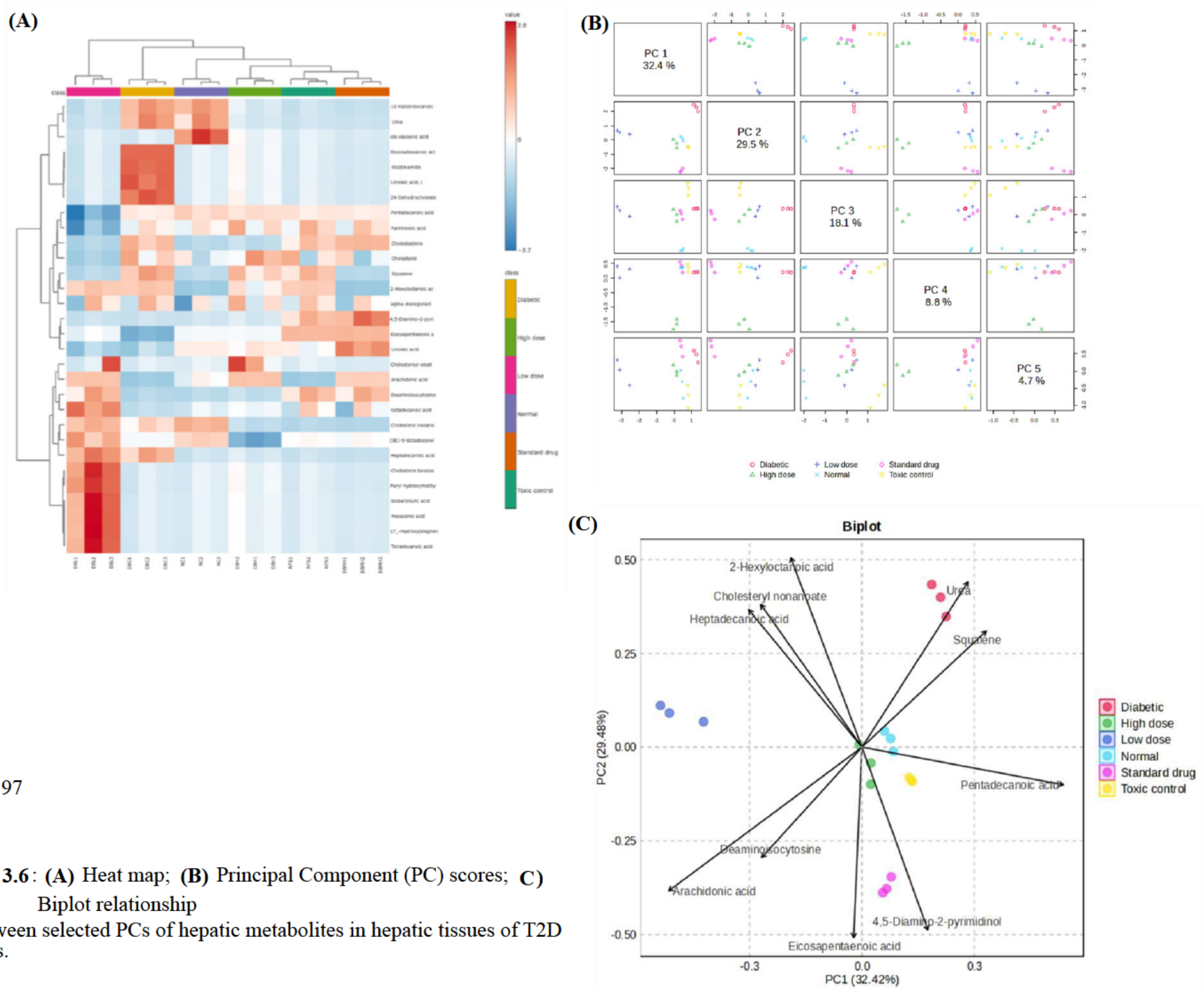
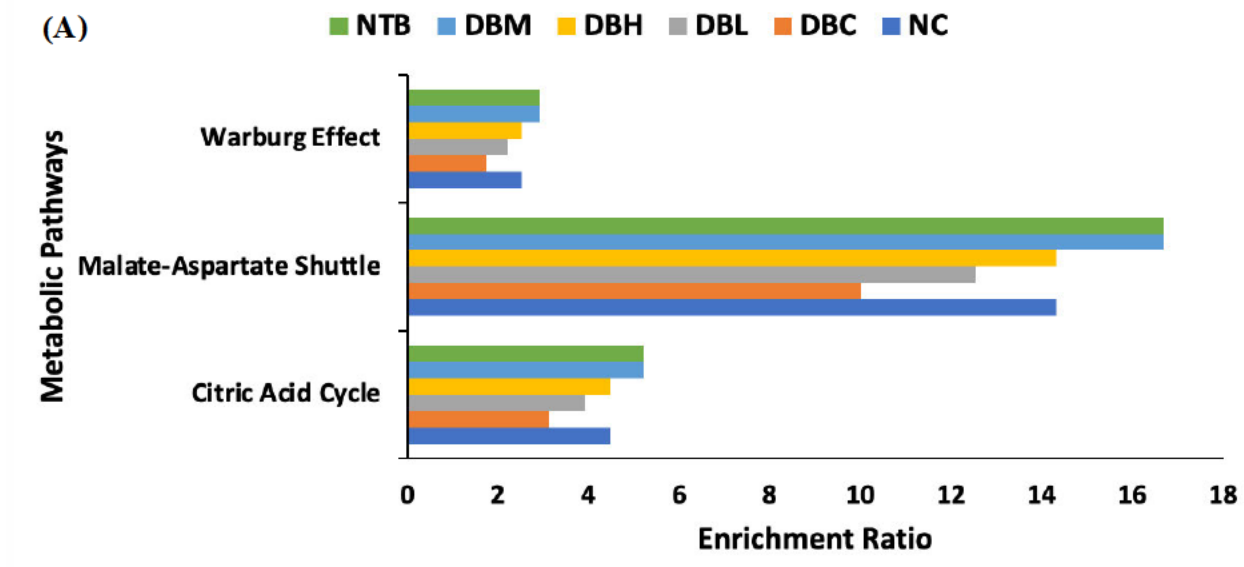


Figure 3.6: **(A)** Heat map; **(B)** Principal Component (PC) scores; **C)** Biplot relationship between selected PCs of hepatic metabolites in hepatic tissues of T2D rats.

Pathway enrichment analysis of hepatic metabolites revealed alterations in glucose metabolism as depicted by the suppressed enrichment ratios of citric acid cycle, malate-aspartate shuttle, and Warburg effect pathways (Figure 3.7A and Table 3.2). These pathways were restored following treatment with both doses of SPs.

Lipid metabolic pathways were also suppressed following induction of T2D (Figure 3.7B and Table 3.2). This is was accompanied by upregulation of steroid biosynthesis, with concomitant inactivation of arachidonic acid metabolism, and activation of propanoate metabolism. Both doses of SPs reactivated arachidonic acid metabolism, with the low dose further activating androgen and estrogen metabolism and fatty acid biosynthesis pathways.

Induction of T2D further altered amino acid metabolism as depicted by suppression of the enrichment ratios and downregulations of the relevant pathways as shown in Figures 3.7C and Table 3.2. Except for d-arginine and d-ornithine metabolism, both doses of SPs upregulated the pathways.



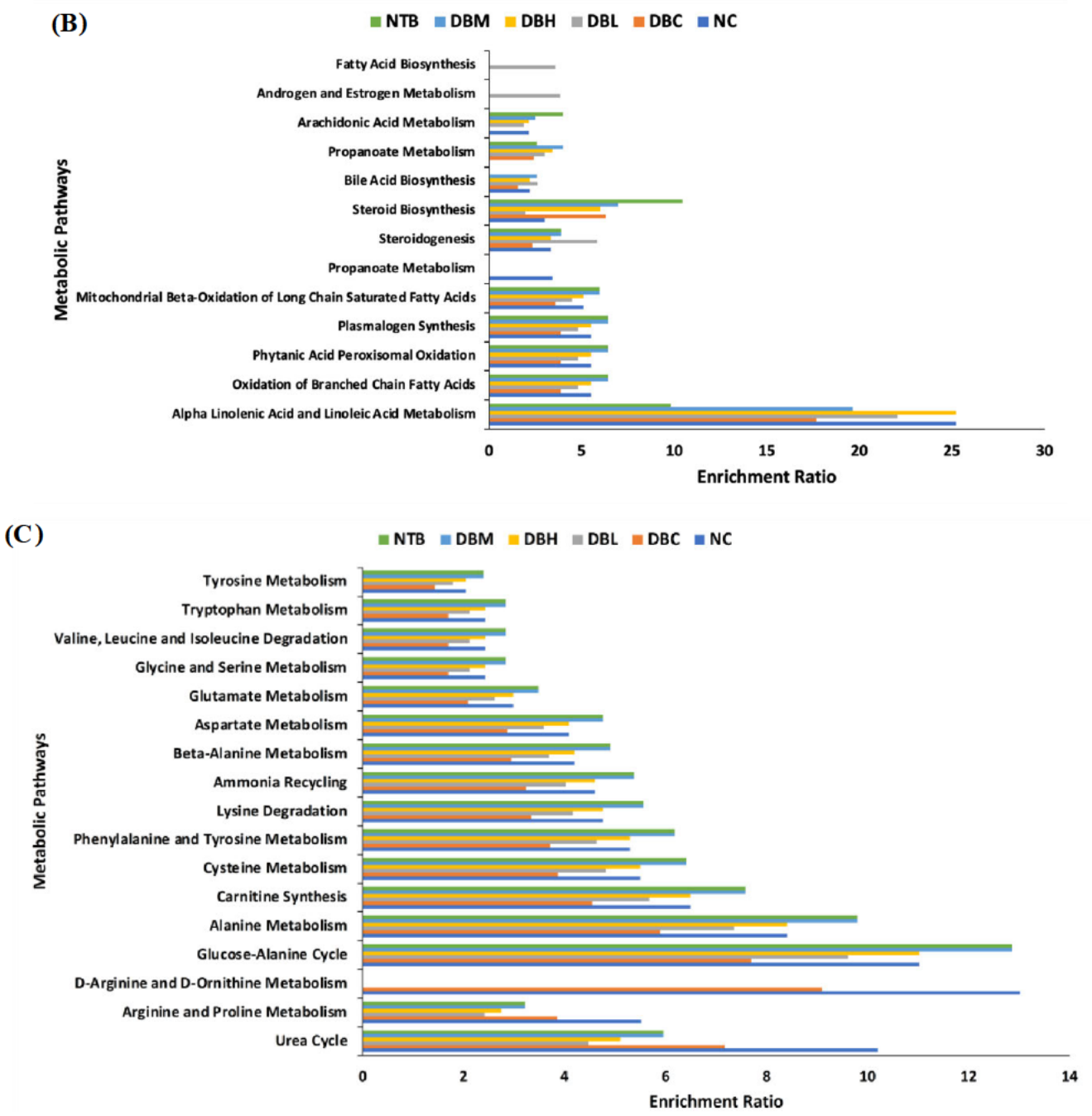


Figure 3.7: Pathway enrichment of hepatic metabolites for (A) glucose, (B) amino acids, and (C) lipid metabolism in hepatic tissues of Type-2 Diabetic rats.

Table 3.2: Pathway of identified hepatic metabolites of T2D rats.

Metabolism	Pathways	NC	DBC	DBL	DBH	DBM	NTB
Glucose metabolism	Citric Acid Cycle	✓	↓	↑	↑	↑	↑
	Malate-Aspartate Shuttle	✓	↓	↑	↑	↑	↑
	Warburg Effect	✓	↓	↑	↑	↑	↑
Lipid Metabolism	Alpha Linolenic Acid and Linoleic Acid Metabolism	✓	↓	↑	↑	↑	↑
	Oxidation of Branched Chain Fatty Acids	✓	↓	↑	↑	↑	↑
	Phytanic Acid Peroxisomal Oxidation	✓	↓	↑	↑	↑	↑
	Plasmalogen Synthesis	✓	↓	↑	↑	↑	↑
	Mitochondrial Beta-Oxidation of Long Chain Saturated Fatty Acids	✓	↓	↑	↑	↑	↑
	Steroidogenesis	✓	↓	↑	↑	↑	↑
	Steroid Biosynthesis	✓	↑	↓	↑	↑	↑
	Bile Acid Biosynthesis	✓	↓	↑	↑	↑	✗
	Propanoate Metabolism	✗	✓	↑	↑	↑	—
	Arachidonic Acid Metabolism	✓	✗	↓	↑	↑	↑
	Androgen and Estrogen Metabolism	✗	✗	✓	✗	✗	✗
	Fatty Acid Biosynthesis	✗	✗	✓	✗	✗	✗

	Carnitine Synthesis	✓	↓	↑	↑	↑	↑
Amino acid metabolism	Urea Cycle	✓	↓	↓	↓	↓	↓
	Arginine and Proline Metabolism	✓	↓	↓	↓	↓	↓
	D-Arginine and D-Ornithine Metabolism	✓	↓	✗	✗	✗	✗
	Glucose-Alanine Cycle	✓	↓	↑	↑	↑	↑
	Alanine Metabolism	✓	↓	↑	↑	↑	↑
	Cysteine Metabolism	✓	↓	↑	↑	↑	↑
	Phenylalanine and Tyrosine Metabolism	✓	↓	↑	↑	↑	↑
	Lysine Degradation	✓	↓	↑	↑	↑	↑
	Ammonia Recycling	✓	↓	↑	↑	↑	↑
	Beta-Alanine Metabolism	✓	↓	↑	↑	↑	↑
	Aspartate Metabolism	✓	↓	↑	↑	↑	↑
	Glutamate Metabolism	✓	↓	↑	↑	↑	↑
	Glycine and Serine Metabolism	✓	↓	↑	↑	↑	↑
	Valine, Leucine and Isoleucine Degradation	✓	↓	↑	↑	↑	↑
	Tryptophan Metabolism	✓	↓	↑	↑	↑	↑

	Tyrosine Metabolism	✓	↓	↑	↑	↑	↑

✓ = present; ↓ = downregulated; ↑ = upregulated; ✗ = inactivated; — = no change

3.4 Discussion

Diabetic hepatopathy has been implicated as a contributor to T2D mediated mortality, and its increasing prevalence is a major concern to health practitioners (Islam et al. 2020; Mallet et al. 2022; Mobasheri et al. 2023; Tolman et al. 2007). Although polysaccharides have been reported for their therapeutic effects on diabetic hepatopathy, there is a dearth of information on the therapeutic effect of SPs. In the present study, we investigated the effect of SPs from *E. maxima* on diabetic hepatopathy in T2D rats.

Abnormal elevated hepatic gluconeogenesis has been linked with diabetic hepatopathy, contributing to hyperglycemia in diabetes (Barroso et al. 2024; Zhang et al. 2019). Incessant glycogenolysis, leading to elevated hyperglycemia has also been reported in diabetic hepatopathy particularly in T2D (Jiang et al. 2020; Rines et al. 2016). These are demonstrated in the present study by the elevated hepatic activities of fructose-1,6-biphosphatase, glucose 6-phosphatase and glycogen phosphorylase in the DBC group (Figure 3.1). Fructose-1,6-biphosphatase and glucose 6-phosphatase are key enzymes in gluconeogenesis, as the former catalyzes the conversion of fructose-1,6-biphosphate to fructose-6-phosphate, while the latter catalyzes the last step which converts glucose-6-phosphate to glucose. Glycogen phosphorylase is a major enzyme in glycogenolysis, as it catalyses the release of glucose-1-phosphate from glycogen. The glucose-1-phosphate is the converted to glucose-6-phosphate. Therefore, the elevated activities of these enzymes indicate glycogenolysis and gluconeogenesis on induction of T2D. This is further corroborated by suppression of citric acid cycle, malateaspartate shuttle and Warburg effect (Tables 3.2 and Figure 3.7A), which are involved in aerobic glucose metabolism and ATP production. Thus, the suppression of these pathways, with concomitant incessant gluconeogenesis and

glycogenolysis, suggest depleted hepatic ATP levels which is a hallmark of diabetic hepatopathy (Koliaki and Roden 2013; Schmid et al. 2011). The decreased activities of these enzymes and upregulation of the aerobic glucose metabolism pathways, following treatment with SPs, indicate metabolic switch towards glycogenesis, glycolysis and the citric acid cycle. This suggests decreased and improved hepatic levels of glucose and ATP, respectively. Inhibition of gluconeogenic and glycogenolytic enzymes have been suggested as a therapeutic strategy in the management of diabetic hepatopathy (Rines et al. 2016), thus, suggesting the therapeutic potential of the SPs in modulating glucose homeostasis in T2D. Excess glucose arising from incessant gluconeogenesis and glycogenolysis can be channeled to other metabolic pathways to generate toxic metabolites, as well as free radicals, which have been implicated in hyperglycemia mediated toxicity. Excess glucose is enolized to enediol radical in a transition-metal dependent reaction (Ahmed 2005; González et al. 2023). The generated enediol radical is further reduced to superoxide anion ($O_2^{\cdot -}$) and reactive ketoaldehydes radicals, which overwhelms the cells' antioxidant system to cause oxidative stress (Snezhkina et al. 2019). Superoxide dismutase reduces the generated $O_2^{\cdot -}$ to hydrogen peroxide (H_2O_2), which if not reduced by glutathione peroxidase and catalase to water (H_2O) and O_2, will be further broken down to hydroxyl radicals ($OH^{\cdot}$) via the Fenton reaction (Meyerstein 2021). Thus, the suppressed hepatic activities of SOD and catalase in DBC group (Figures 3.2A and 3.2B), suggest elevated hepatic levels of $O_2^{\cdot -}$ and $OH^{\cdot}$ **(**from H_2O_2). These elevations and the suppressed enzymes activities indicate oxidative stress which have been implicated in the pathophysiology of diabetic hepatopathy. It also corresponds with previous studies which reported reduced activities of SOD and catalase in hepatic tissues of diabetic rats (Adeghate et al. 2021; Erukainure et al. 2021). The increased activities of the enzymes

following treatment with SPs, therefore indicates an antioxidant effect. It also suggests suppressed levels of $O_2^{\cdot-}$ and the reduction of H_2O_2 to H_2O and O_2.

Excess glucose can also be channelled to the polyol pathway, where it is reduced to sorbitol by aldose reductase. Sorbitol dehydrogenase then oxidizes sorbitol to fructose (Erukainure and Chukwuma 2024). In the first step of the pathway, aldose reductase arrests the oxidation of NADH to NAD+, leading to alteration of glutathione metabolism which can induce oxidative stress (Brownlee 2005). This is demonstrated in the present study by the decreased GSH level in hepatic tissues of diabetic rats (Figure 3A). This is further depicted by the suppressed activities of glutathione reductase, glutathione peroxidase and glutathione s-transferase (GST) (Figures 3.3B – D). Glutathione reductase reduces oxidized glutathione (GSSG) to GSH, while glutathione peroxidase oxidizes GSH to GSSG (Couto et al. 2016; Erukainure and Chukwuma 2024). Their suppressed activities depict distortion in GSH homeostasis, which further portrays an occurrence of hepatic oxidative stress following induction of T2D. Furthermore, the reduced GST activity depicts elevated ROS levels. The elevated activities of these enzymes and GSH level following treatment with SPs, indicate improved hepatic glutathione metabolism and antioxidant activities, which are important therapeutic strategies in the management of diabetic hepatopathy (Dawi et al. 2024; Nguyen et al. 2025).

Hydroxyl radicals from the breakdown of H_2O_2, owing to suppressed activities of catalase and glutathione peroxidase activities, attacks the membrane lipid, setting up a lipid peroxidation chain reaction (Ighodaro and Akinloye 2018; Jena et al. 2023). The products of lipid peroxidation are the 3-carbon dialdehyde species, malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) (Su et al. 2019). Therefore, the elevated MDA level in the hepatic tissues of untreated diabetic rats (Figure 3.4), indicates an occurrence of lipid peroxidation. This correlates with the altered

glutathione metabolism and decreased antioxidant enzymes activities, further confirming oxidative stress on induction of T2D. It also corroborates previous studies on elevated lipid peroxidation in diabetic hepatopathy, and has been implicated in its pathophysiology and cell death (de Souza Bastos et al. 2016; Shabalala et al. 2022). The depleted hepatic MDA levels in T2D rats treated with SPs, indicates an antioxidant effect. This correlates with the improved antioxidant enzymes activities and glutathione metabolism. Thus, further suggesting the antioxidant therapeutic effect of SPs against diabetic hepatopathy in T2D.

Elevated hepatic acetylcholinesterase activity leading to decreased cellular level of acetylcholine has been implicated in low-grade systemic inflammation in diabetic hepatopathy (Das 2012; Mahmood et al. 2021). The elevated hepatic acetylcholinesterase activity following induction of T2D (Figure 3.5), therefore, indicates reduced hepatic level of acetylcholine. Thus, suggesting an occurrence of inflammation. This correspond with previous studies on elevated hepatic acetylcholinesterase activity in T2D (Erukainure et al. 2021). The suppressed acetylcholinesterase activity following treatment with SPs, indicates elevated cellular levels of acetylcholine. Thereby suggesting improved an anti-inflammatory effect. Acetylcholine initiates the cholinergic antiinflammatory pathway by binding to the α-7 subunits of nicotinic acetylcholine receptors, thus suppressing the release of cytokines (Bondok et al. 2013).

Distorted metabolism of hepatic lipid and its metabolites leading to hyperlipidemia and lipotoxicity have been implicated in the pathophysiology of diabetic hepatopathy (Gluchowski et al. 2017). It is characterized by imbalances in lipid metabolism arising from excessive fat accumulation, alterations in fatty acid oxidation and hepatic steroid metabolism, leading to hepatocyte injury, oxidative stress and inflammation (Mendez-Sanchez et al. 2018). The alterations in hepatic free fatty acids (FFAs) concentrations, and concomitant generation of additional FFAs,

as well as their disparity on induction of T2D (Table 3.1 and Figures 3.6A – C), indicate hepatic fat accumulation and lipotoxicity. This is further depicted by the downregulation of hepatic lipid metabolic pathways, particularly oxidation of branched chain fatty acids, phytanic acid peroxisomal oxidation, and mitochondrial beta-oxidation of long chain saturated fatty acids pathways (Figure 3.7B and Table 3.2). These can be attributed to the altered FFAs on induction of T2D. Furthermore, the altered hepatic steroid metabolic pathways which includes steroidogenesis, steroid biosynthesis and bile acid biosynthesis, can be attributed the altered cholesteric metabolites including cholesterol. These alterations corroborate previous reports on distorted hepatic lipid metabolism in T2D (Erukainure et al. 2021). The improved hepatic FFAs levels and constituents, with concomitant upregulation of lipid metabolic pathways in diabetic rats treated with SPs, indicate suppressed hepatic lipid accumulation and improved metabolism. These improvements suggest an anti-lipotoxic effect of the polysaccharides.

Altered hepatic amino acid metabolism has been implicated in the pathophysiology of diabetic hepatopathy, with insulin resistance playing an influential role (Cuomo et al. 2022; Wiklund et al. 2018). These alterations lead to changes in blood concentrations of essential amino acids, particularly branched chain amino acids (BCAAs), tyrosine, phenylalanine, and sulfur amino acids, and have been linked to insulin resistance (Adams 2011). This is demonstrated in the present study by the deregulated amino acid metabolic pathways in hepatic tissues of untreated diabetic rats (Figure 3.7C and Table 3.2). These dysregulated pathways can be attributed to the depleted hepatic urea and alpha-ketoglutaric acid levels. They also suggest altered blood levels of amino acids. The upregulated pathways and concomitant elevated levels of urea and alpha-ketoglutaric acid following treatment with SPs, indicates improved amino acid metabolism. This improvement further depicts the therapeutic potential of the polysaccharides against diabetic hepatopathy. The

biochemical and metabolomics outcomes is presented schematically in Figure 3.8, and can be summarized as:

1. **Glycolysis → Pyruvate** where pyruvate is the central metabolite:

   Pyruvate → Acetyl-CoA → TCA Cycle or De novo lipogenesis (1)

   Pyruvate ↔ Alanine (via ALT) → Glucose-Alanine cycle (2)

2. **TCA cycle ↔ Amino acid metabolism**

   intermediates such as α-ketoglutarate are utilized for transamination reactions.

3. **Glucose-Alanine Cycle**

   A. **Muscle**: Pyruvate + NH_3 → Alanine → Liver (3)

   B. **Liver**: Alanine → Pyruvate + NH_3 (urea cycle) (4)

4. **Urea Cycle** removal of nitrogen (via amino acid hydrolysis) utilizing NH_3 and aspartate (from TCA).

5. **De Novo Lipogenesis**

   Acetyl-CoA → Fatty acids (5)

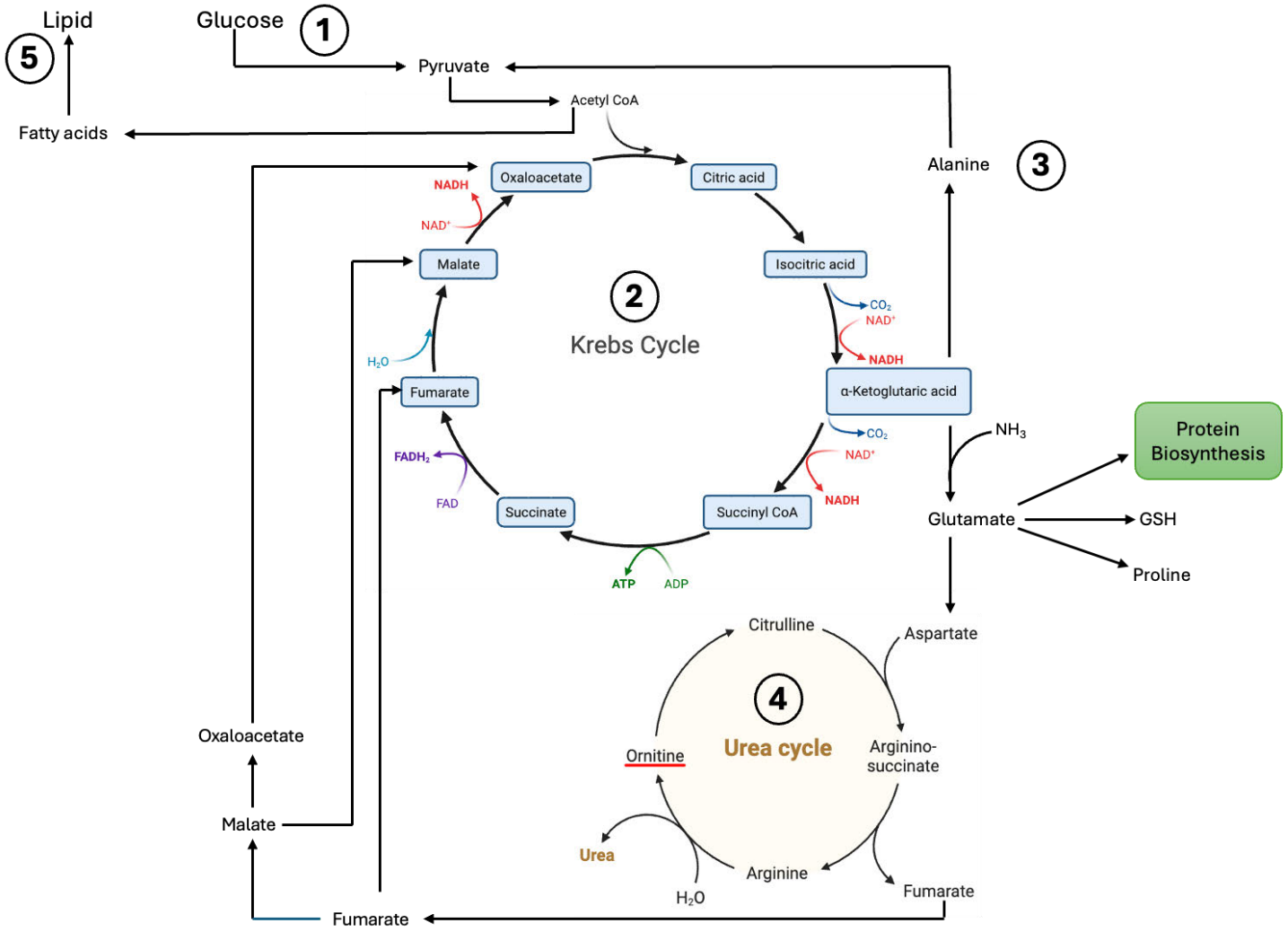


Figure 3.8: Schematic pathway by which Sulphated Polysaccharides may improve protect against diabetic hepatopathy in Type-2 Diabetes. **(1)**: Glycolysis; **(2)**: TCA/Krebs cycle; **(3)**: Glucose-Alanine cycle; **(4)**: Urea cycle; and **(5)** De novo lipogenesis.

3.5 Conclusion

Diabetic hepatopathy involves a complex interplay of metabolic disturbances, encompassing hyperglycemia, altered hepatic steroidogenesis, elevated fat accumulation, dysregulated amino acid metabolism, oxidative stress, and inflammation, ultimately leading to liver damage and dysfunction. Treatment with SPs from *E. maxima* protected against diabetic hepatopathy in T2D rats by improving glucose and amino acid metabolisms, while concomitantly attenuating lipotoxicity, oxidative stress and inflammation in liver tissues. However, the translational relevance of the therapeutic potentials of these SPs needs to be investigated in clinical trials.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported by a grant from Technology Innovation Agency (TIA), Durban, South Africa (Grant no. SABDI 16/1072), and Incentives for Rated Researchers by the National Research Foundation (NRF).

Data availability statement

The data in support this research findings are presented in the article.

3.6 Reference

Adams SH (2011) Emerging perspectives on essential amino acid metabolism in obesity and the insulin-resistant state. Advances in nutrition 2(6):445-456

Adeghate E, D'Souza CM, Saeed Z, et al. (2021) Nociceptin increases antioxidant expression in the kidney, liver and brain of diabetic rats. Biology 10(7):621

Ahmed N (2005) Advanced glycation endproducts—role in pathology of diabetic complications. Diabetes research and clinical practice 67(1):3-21

Barroso E, Jurado-Aguilar J, Wahli W, Palomer X, Vázquez-Carrera M (2024) Increased hepatic gluconeogenesis and type 2 diabetes mellitus. Trends in Endocrinology & Metabolism

Bondok RS, Ahmed MA, Soliman NBE, et al. (2013) The effect of cholinesterase inhibition on liver dysfunction in experimental acute liver failure. The Egyptian Journal of Critical Care Medicine 1(2):51-59

Brownlee M (2005) The pathobiology of diabetic complications: a unifying mechanism. Diabetes 54(6):1615-1625

Couto N, Wood J, Barber J (2016) The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. Free radical biology and medicine 95:27-42

Cuomo P, Capparelli R, Iannelli A, Iannelli D (2022) Role of branched-chain amino acid metabolism in type 2 diabetes, obesity, cardiovascular disease and non-alcoholic fatty liver disease. International journal of molecular sciences 23(8):4325

Das UN (2012) Acetylcholinesterase and butyrylcholinesterase as markers of low-grade systemic inflammation. Annals of hepatology 11(3):409-411

Daub CD, Mabate B, Malgas S, Pletschke BI (2020) Fucoidan from Ecklonia maxima is a powerful inhibitor of the diabetes-related enzyme, α-glucosidase. International journal of biological macromolecules 151:412-420

Dawi J, Misakyan Y, Affa S, et al. (2024) Oxidative Stress, Glutathione Insufficiency, and Inflammatory Pathways in Type 2 Diabetes Mellitus: Implications for Therapeutic Interventions. Biomedicines 13(1):18 de Souza Bastos A, Graves DT, de Melo Loureiro AP, et al. (2016) Diabetes and increased lipid peroxidation are associated with systemic inflammation even in well-controlled patients. Journal of Diabetes and its Complications 30(8):1593-1599

Ding Y, Wang S, Lu J (2023) Unlocking the potential: amino acids' role in predicting and exploring therapeutic avenues for type 2 diabetes mellitus. Metabolites 13(9):1017

Diyorbek I (2025) Pathogenesis of chronic complications of type 2 diabetes mellitus and ways of their correction. Web of Medicine: Journal of Medicine, Practice and Nursing 3(2):15-17

Erukainure OL, Chukwuma CI (2024) African walnut (Plukenetia conophora) oil promotes glucose uptake while improving energy metabolism and steroidogenesis and maintaining surface architecture in rat testes. Frontiers in Nutrition 11:1505453

Erukainure OL, Sanni O, Salau VF, Koorbanally NA, Islam MS (2021) Cola nitida (kola nuts) attenuates hepatic injury in type 2 diabetes by improving antioxidant and cholinergic dysfunctions and dysregulated lipid metabolism. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders) 21(4):688-699

Gluchowski NL, Becuwe M, Walther TC, Farese Jr RV (2017) Lipid droplets and liver disease: from basic biology to clinical implications. Nature reviews Gastroenterology & hepatology 14(6):343-355

González P, Lozano P, Ros G, Solano F (2023) Hyperglycemia and oxidative stress: An integral, updated and critical overview of their metabolic interconnections. International Journal of Molecular Sciences 24(11):9352

Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW (2016) Non-alcoholic fatty liver disease and diabetes. Metabolism 65(8):1096-1108

IDF (2025) IDF Diabetes Atlas. In: International Diabetes Federation. https://diabetesatlas.org/resources/idf-diabetes-atlas-2025/ Accessed April 17 2025

Ighodaro O, Akinloye O (2018) First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria journal of medicine 54(4):287-293

Islam S, Rahman S, Haque T, Sumon AH, Ahmed AM, Ali N (2020) Prevalence of elevated liver enzymes and its association with type 2 diabetes: A cross-sectional study in Bangladeshi adults. Endocrinology, diabetes & metabolism 3(2):e00116

Jena AB, Samal RR, Bhol NK, Duttaroy AK (2023) Cellular Red-Ox system in health and disease: The latest update. Biomedicine & Pharmacotherapy 162:114606

Jia R-B, Wu J, Li Z-R, et al. (2020) Structural characterization of polysaccharides from three seaweed species and their hypoglycemic and hypolipidemic activities in type 2 diabetic rats. International journal of biological macromolecules 155:1040-1049

Jiang S, Young JL, Wang K, Qian Y, Cai L (2020) Diabetic-induced alterations in hepatic glucose and lipid metabolism: the role of type 1 and type 2 diabetes mellitus. Molecular medicine reports 22(2):603-611

Koliaki C, Roden M (2013) Hepatic energy metabolism in human diabetes mellitus, obesity and non-alcoholic fatty liver disease. Molecular and Cellular Endocrinology 379(1-2):35-42 Lee H-

G, Je J-G, Hwang J, et al. (2021) Comparision of antioxidant and anti-inflammatory activities of enzyme assisted hydrolysate from Ecklonia maxima blades and stipe. Fisheries and Aquatic Sciences 24(5):197-206

Ma Y, Lee G, Heo S-Y, Roh Y-S (2021) Oxidative stress is a key modulator in the development of nonalcoholic fatty liver disease. Antioxidants 11(1):91

Mahmood AA, Zainal AA, Khalaf MM (2021) Cholinesterase activity in non-alcoholic-fatty liver disease in diabetic patients taking oral antidiabetic drugs. Pharmacology 25

Mallet V, Parlati L, Martinino A, et al. (2022) Burden of liver disease progression in hospitalized patients with type 2 diabetes mellitus. Journal of Hepatology 76(2):265-274

Mendez-Sanchez N, Cruz-Ramon VC, Ramirez-Perez OL, Hwang JP, Barranco-Fragoso B, Cordova-Gallardo J (2018) New aspects of lipotoxicity in nonalcoholic steatohepatitis. International journal of molecular sciences 19(7):2034

Meyerstein D (2021) Re-examining Fenton and Fenton-like reactions. Nature Reviews Chemistry 5(9):595-597

Mobasheri L, Ahadi M, Namdar AB, et al. (2023) Pathophysiology of diabetic hepatopathy and molecular mechanisms underlying the hepatoprotective effects of phytochemicals. Biomedicine & Pharmacotherapy 167:115502

Nagahawatta D, Liyanage N, Jayawardhana H, Lee H-G, Jayawardena TU, Jeon Y-J (2022) Antifine dust effect of fucoidan extracted from Ecklonia maxima leaves in macrophages via inhibiting inflammatory signaling pathways. Marine Drugs 20(7):413

Nguyen MT, Lian A, Guilford FT, Venketaraman V (2025) A Literature Review of Glutathione Therapy in Ameliorating Hepatic Dysfunction in Non-Alcoholic Fatty Liver Disease. Biomedicines 13(3):644

Olasehinde TA, Mabinya LV, Olaniran AO, Okoh AI (2019) Chemical characterization, antioxidant properties, cholinesterase inhibitory and anti-amyloidogenic activities of sulfated polysaccharides from some seaweeds. Bioactive Carbohydrates and Dietary Fibre 18:100182 Pillay LR, Olasehinde TA, Olofinsan K, et al. (2025) The impact of sulphated polysaccharides from Ecklonia maxima on carbohydrate hydrolyzing enzymes, muscle glucose uptake and intestinal glucose absorption ex vivo. Algal Research 85:103831

Ren S, Wang J, Wang Y, et al. (2025) Oxidative stress and type 2 diabetes: a review of lactic acid bacteria as potential prophylactic and therapeutic interventions. Food Science and Biotechnology:1-14

Rines AK, Sharabi K, Tavares CD, Puigserver P (2016) Targeting hepatic glucose metabolism in the treatment of type 2 diabetes. Nature reviews Drug discovery 15(11):786-804

Schmid AI, Szendroedi J, Chmelik M, Krššák M, Moser E, Roden M (2011) Liver ATP synthesis is lower and relates to insulin sensitivity in patients with type 2 diabetes. Diabetes care 34(2):448453

Shabalala SC, Johnson R, Basson AK, et al. (2022) Detrimental effects of lipid peroxidation in type 2 diabetes: Exploring the neutralizing influence of antioxidants. Antioxidants 11(10):2071

Snezhkina AV, Kudryavtseva AV, Kardymon OL, et al. (2019) ROS generation and antioxidant defense systems in normal and malignant cells. Oxidative medicine and cellular longevity 2019(1):6175804

Su L-J, Zhang J-H, Gomez H, et al. (2019) Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. Oxidative medicine and cellular longevity 2019(1):5080843

Tolman KG, Fonseca V, Dalpiaz A, Tan MH (2007) Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. Diabetes care 30(3):734-743 Westcott

F, Dearlove DJ, Hodson L (2024) Hepatic fatty acid and glucose handling in metabolic disease: potential impact on cardiovascular disease risk. Atherosclerosis 394:117237

Wiklund P, Zhang X, Pekkala S, Autio R, Kong L, Yang Y (2018) Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. Sci Rep. 2016; 6: 1–11. American Journal of Epidemiology 187(8):1662-1669

Zhang X, Yang S, Chen J, Su Z (2019) Unraveling the regulation of hepatic gluconeogenesis. Frontiers in Endocrinology 9:802

Zhong Q-W, Zhou T-S, Qiu W-H, et al. (2021) Characterization and hypoglycemic effects of sulfated polysaccharides derived from brown seaweed Undaria pinnatifida. Food Chemistry 341:128148

Zhu H, Bai M, Xie X, et al. (2022) Impaired amino acid metabolism and its correlation with diabetic kidney disease progression in type 2 diabetes mellitus. Nutrients 14(16):3345

CHAPTER 4

Sulphated Polysaccharides from *Gracilaria gracilis* (Red Seaweed) Mitigates Diabetic Hepatopathy by Ameliorating Glucolipotoxicity and Amino Acid Dysmetabolism, while Exacerbating Antioxidative Activities in Hepatic Tissues of Type 2 Diabetic Rats

[1]Lethiwe B. Mpungose, [1]Tosin A. Olasehinde, [1,2]Ochuko L. Erukainure, [2]Md. Shahidul Islam, [1]Ademola O. Olaniran*

1. Department of Microbiology, School of Life Sciences, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa.
2. Department of Biochemistry, School of Life Sciences, University of Kwazulu-Natal, Westville Campus, Durban 4000, South Africa.

***Correspondence:** olanirana@ukzn.ac.za

Abstract

Diabetic hepatopathy is among the complications of type 2 diabetes (T2D) and has been reported for its role in immortality and morbidities associated with the disease. Seaweeds are the major sources of sulphated polysaccharides (SPs), with reported antidiabetic properties. The present study investigated the therapeutic effect of SPs from *Gracilaria gracilis* on diabetic hepatopathy in rats with T2D. SPs were administered at 150 and 300 mg/Kg bodyweight (bw) to two groups of T2D rats, respectively. The positive group and negative group were administered water, while metformin served as the standard antidiabetic drug. The toxicology group consisted of normal rats administered 300 mg/Kg bw SPs. Induction of T2D led to the deletion of glutathione level, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione Stransferase activities, while concomitantly elevating fructose-1.6-biphosphatase, glucose 6phosphatase, glycogen phosphorylase, acetylcholinesterase activities, and malondialdehyde level in hepatic tissues. Glucose, lipid and amino acid metabolic pathways, and their metabolites were also dysregulated on induction of T2D. Treatment with SPs led to the reversal of the glutathione and malondialdehyde levels, and enzymes activities, while concomitantly improving glucose, lipid and amino acid metabolisms. Thus, indicating that SPs from *G. gracilis* protects against diabetic hepatopathy in T2D by attenuating lipotoxicity, oxidative stress and inflammation, with concomitant improved glucose, lipid and amino acid metabolisms.

Keywords: Glucolipotoxicity; Hepatopathy; Oxidative stress; Red seaweeds; Sulphated polysaccharides; and Type 2 Diabetes

4.1 Introduction

Diabetes is a metabolic disorder characterized by hyperglycemia arising from inability of the pancreatic β-cells to secrete insulin and/or inability of the body to utilize secreted insulin. The former is known as type 1 diabetes (T1D), while the latter, type 2 diabetes (T2D). Type 2 diabetes accounts for over 90% of all diabetes-related morbidities and mortalities, thus making it the most prominent of all diabetes types (IDF 2025). It is characterized by insulin resistance and dysfunction in pancreatic β-cells, leading to hyperglycemia. Excessive hyperglycemia has been implicated in production of reactive oxygen species (ROS) and free radicals, which cause a shift in the body's oxidant-antioxidant balance. This imbalance is termed oxidative stress and has been linked in the pathophysiology of T2D complications (Diyorbek 2025; Ren et al. 2025).

Diabetic hepatopathy is among the complications associated with T2D, and has been attributed to the role of hepatic tissues in glucose homeostasis (Erukainure et al. 2021).. It is a non-alcoholic fatty liver disease (NAFLD) and its spectrum, including hepatosclerosis, glycogenic hepatopathy, abnormal liver enzymes, cirrhosis, hepatitis C infection and acute liver failure, associated with diabetes (Islam et al. 2020; Mobasheri et al. 2023; Tolman et al. 2007).

T2D-induced insulin resistance alters hepatic glucose uptake and utilization, ultimately leading to an increase in hepatic output, and further contributing blood glucose spike (Erukainure et al. 2021). Alterations to lipid metabolic pathways and their associated metabolites have also been linked to diabetic hepatopathy (Erukainure et al. 2021; Hazlehurst et al. 2016). The condition is reportedly caused by a heightened uptake of hepatic fatty acids, an increase in *de novo* lipogenesis, and a reduction in the secretion of very low-density lipoprotein (VLDL), resulting in hypertriglyceridemia and hypercholesteremia (Mobasheri et al. 2023; Westcott et al. 2024).

Additionally, perturbation in amino acid metabolism exacerbates hepatic glucose production, which fuels the tissues' insensitivity to insulin, ultimately resulting in glucotoxicity (Ding et al. 2023; Zhu et al. 2022). The interplay of these pathophysiological activities results to glucolipotoxicity, with concomitant generation of ROS, ultimately leading to hepatic oxidative stress (Ma et al. 2021).

The role of sulphated polysaccharides (SPs) from seaweeds in the treatment of T2D and its complications have been reported (Jia et al. 2020; Zhong et al. 2021). These medicinal properties have been ascribed to their ability to scavenge free radicals, reduction carbohydrate digestive enzymes, improve hepatic lipid metabolism, improve hepatic functions and glucose metabolism, and promote muscle glucose uptake (Murakami et al. 2021; Pillay et al. 2025; Ponce and Stortz 2020; Wen et al. 2017; Zhang et al. 2022). *Gracilaria gracilis* is a red seaweed and belongs to the Gracilaria genus. The monosaccharide composition of its SPs have been reported to consist of glucose, mannuronic acid, galactose, arabinose, xylose, and glucuronic acid (185 : 141 : 21 : 12 : 1 : 142) (Pillay et al. 2024). The antidiabetic properties of *G. gracilis* SPs have been demonstrated by their ability to promote muscle glucose uptake, and inhibition of carbohydrate digestive enzyme activities and intestinal glucose absorption (Pillay et al. 2024).

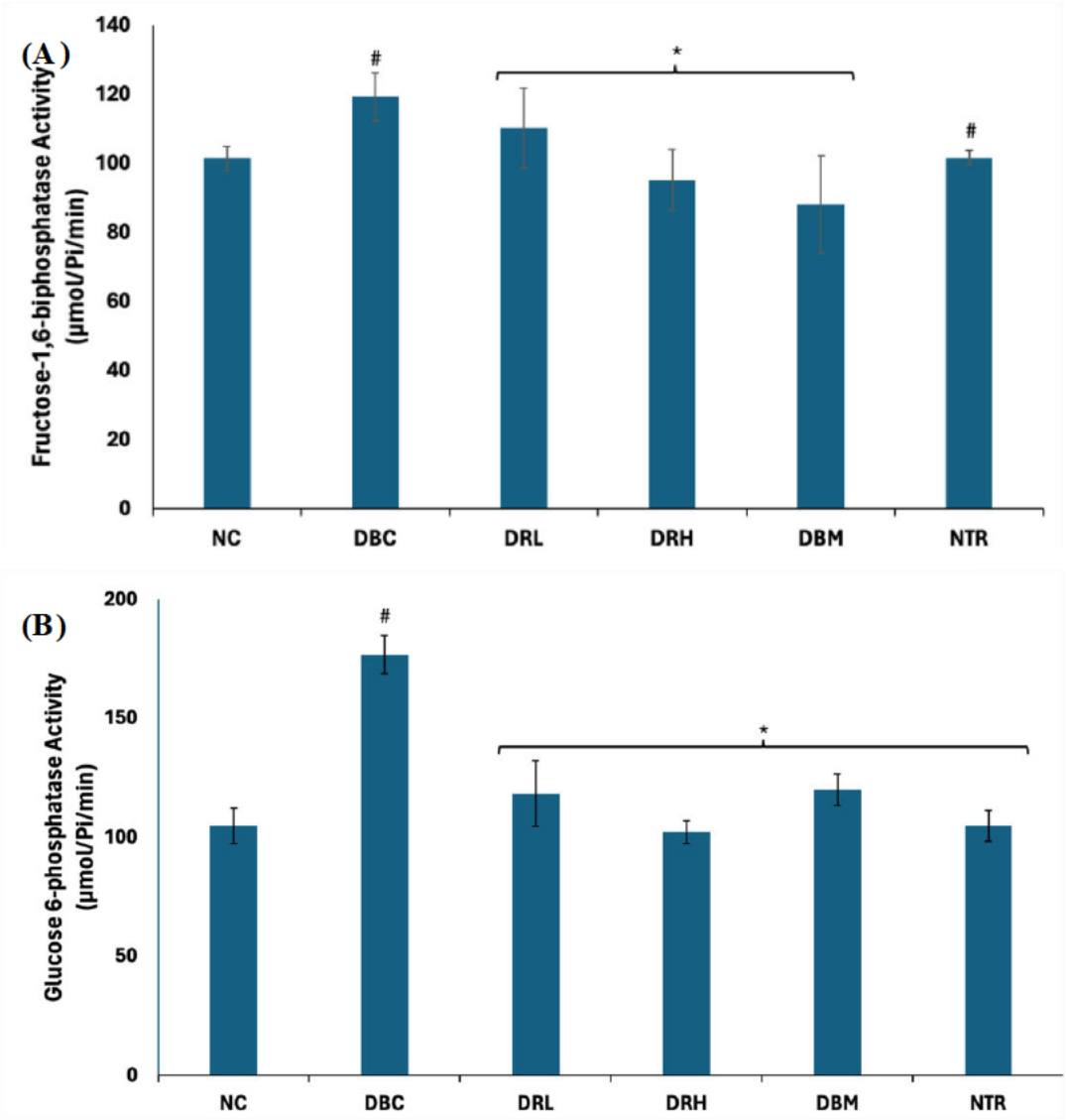
However, SPs from *G. gracilis* have not been fully investigated for their effect on diabetic hepatopathy. Therefore, the present study was aimed at investigating the effect of the SPs on. diabetic hepatopathy, by exploring its ability to improve hepatic metabolism of glucose, lipid and amino acid as well as its ameliorative effect on oxidative stress in hepatic tissues of rats with T2D.

4.2 Materials and Methods

Kindly refer to sections 2.1, 2.2, and 2.4 – 2.15 in Chapter 2.

4.3 Results

Hepatic activities of fructose-1,6-biphosphatase, glucose 6-phosphatase and glycogen phosphorylase were significantly high ($p<0.05$) following the induction of T2D as shown in Figures 4.1A – 4.1C. Treatment with SPs significantly improved enzyme activities when compared with negative control (NC) group.



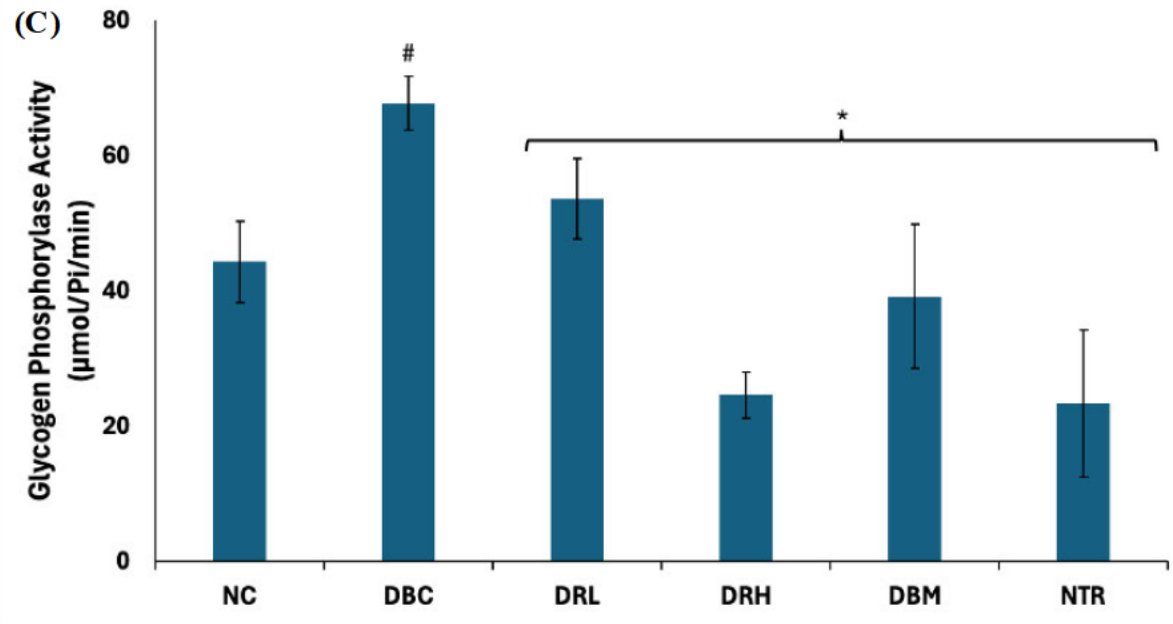


Figure 4.1: Effect of SPs on (A) fructose-1,6-biphosphatase, (B) glucose 6-phosphatase, and (C) glycogen phosphorylase activities in hepatic tissues of T2D rats. Values = mean±SD; n=6. *Statistically significant ($p<0.05$) to DBC; #statistically ($p<0.05$) significant to NC).

As shown in Figures 4.2A and 4.2B, induction of T2D led to significant ($p<0.05$) depletion in hepatic activities of SOD and catalase. These activities were significantly ($p<0.05$) elevated following treatment with SPs and compared favorably with NC.

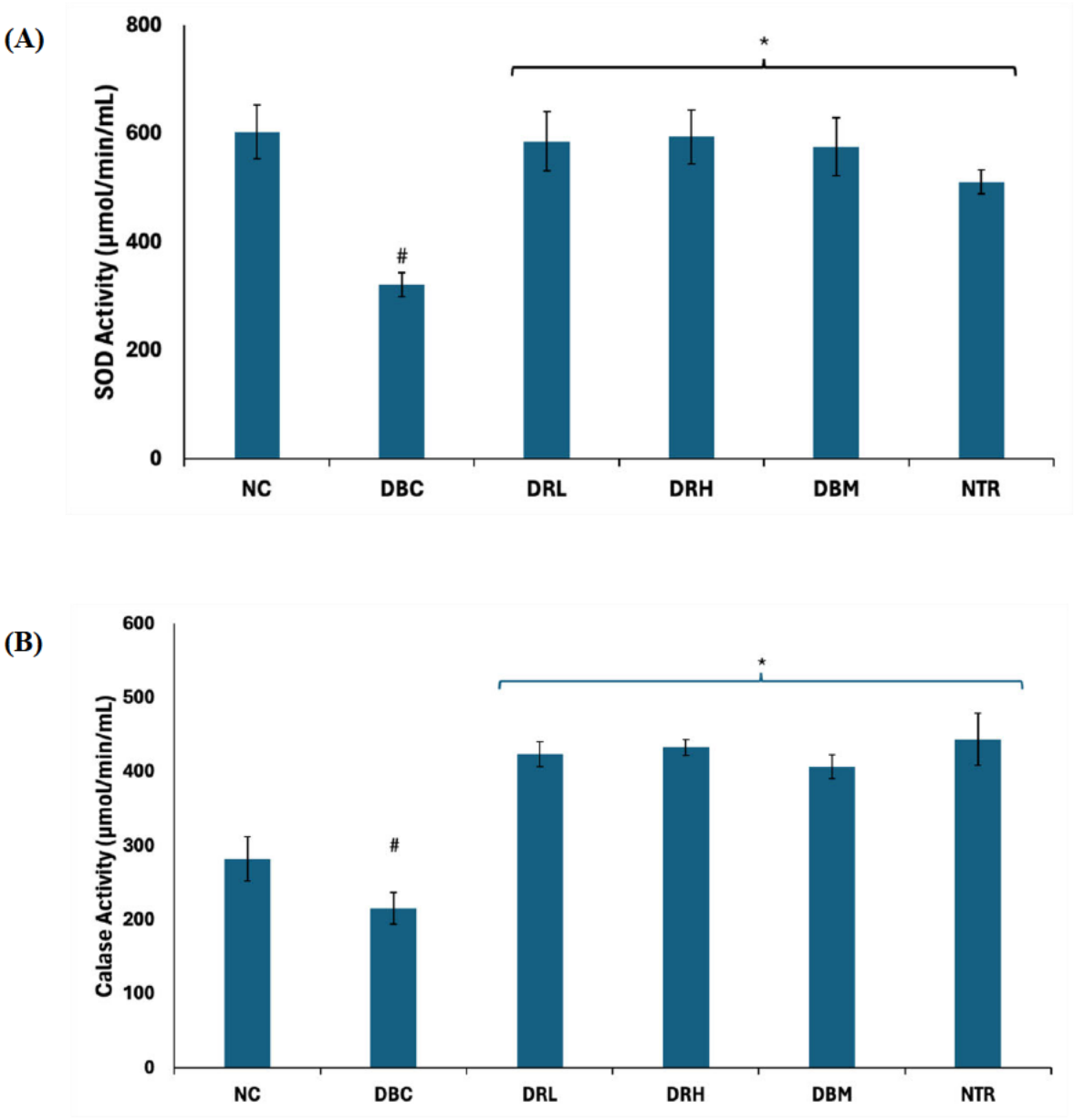


Figure 4.2: Effect of SPs on (A) SOD, and (B) catalase activities in hepatic tissues of T2D rats. Values = mean±SD; n=6. *Statistically significant ($p<0.05$) to DBC; #statistically ($p<0.05$) significant to NC).

There was a significant ($p<0.05$) depletion in GSH level, while concomitantly depleting glutathione reductase, glutathione peroxidase and glutathione S- transferase activities as shown in Figures 4.3A – D. These protein level and enzyme activities were significantly ($p<0.05$) elevated following treatment with SPs and compared favourably with NC.

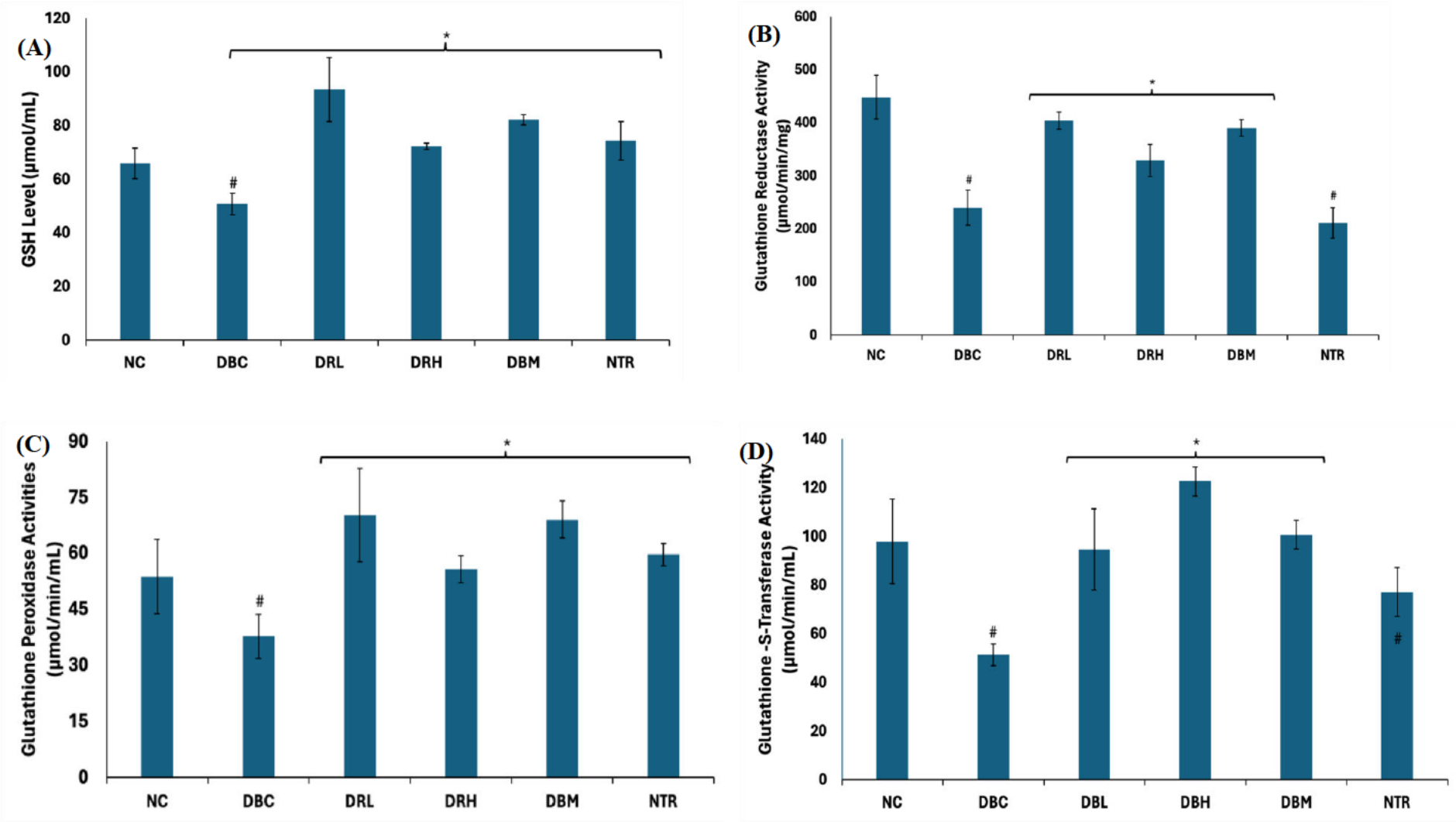


Figure 4.3: Effect of SPs on (A) GSH level, (B) glutathione reductase, (C) glutathione peroxidase, and (D) glutathione-s-transferase activities in hepatic tissues of T2D rats. Values = mean±SD; n=6. *Statistically significant ($p<0.05$) to DBC; #statistically ($p<0.05$) significant to NC).

There was a significant ($p<0.05$) elevation in hepatic MDA level, following the induction of T2D as shown in Figure 4.4. Treatment with SPs significantly ($p<0.05$) reversed the MDA level, and compared favorably with NC.

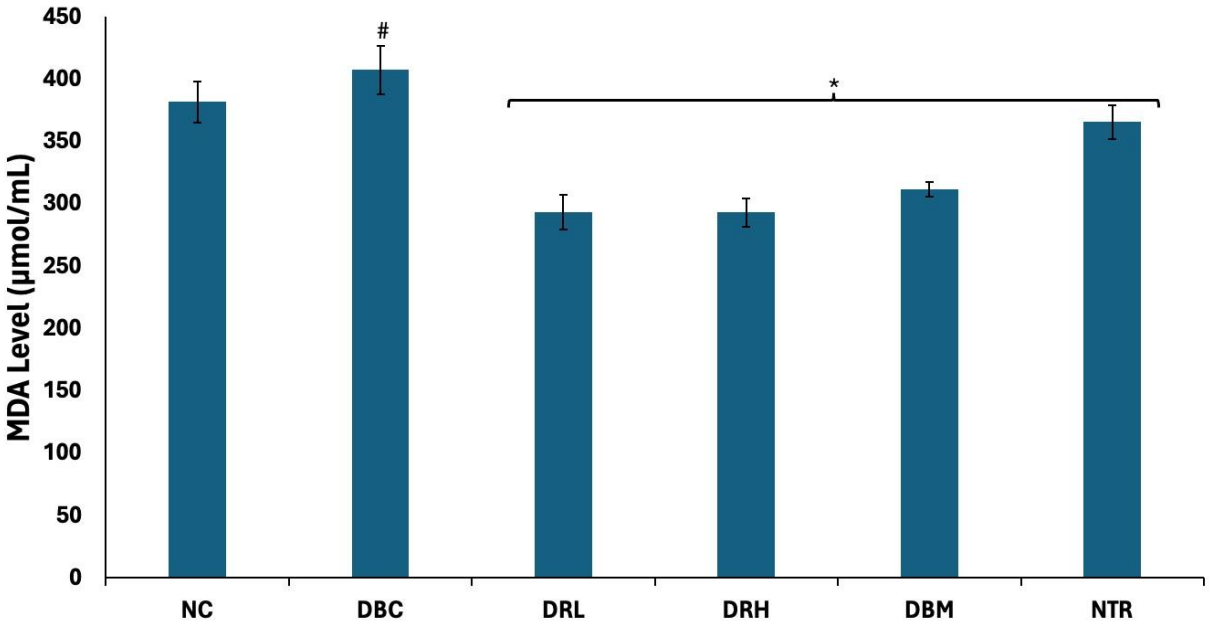


Figure 4.4: Effect of SPs on MDA level in hepatic tissues of T2D rats. Values = mean±SD; n=6. *Statistically significant ($p<0.05$) to DBC; #statistically ($p<0.05$) significant to NC).

As shown in Figure 4.5, induction of T2D led to significant ($p<0.05$) elevated hepatic activity of acetylcholinesterase. The activity was significantly ($p<0.05$) depleted following treatment with SPs, and compared favourably with NC.

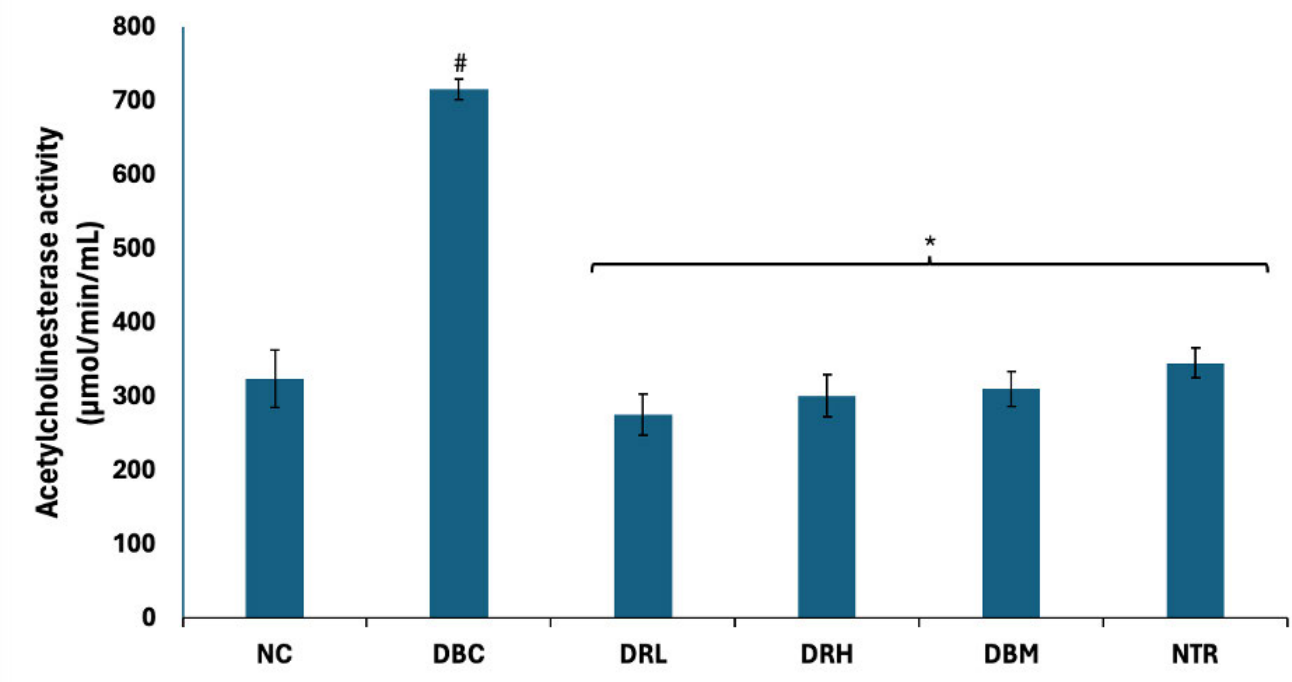


Figure 4.5: Effect of SPs on acetylcholinesterase activity in hepatic tissues of T2D rats. Values = mean±SD; n=6. *Statistically significant ($p<0.05$) to DBC; #statistically ($p<0.05$) significant to NC).

As shown in Figures 4.6, serum levels of AST and ALP were significantly ($p<0.05$) elevated following the induction of T2D. Treatment with SPs led significantly ($p<0.05$) suppressed levels of the hepatic markers, except for AST and ALP levels in DRL and DRH groups, respectively.

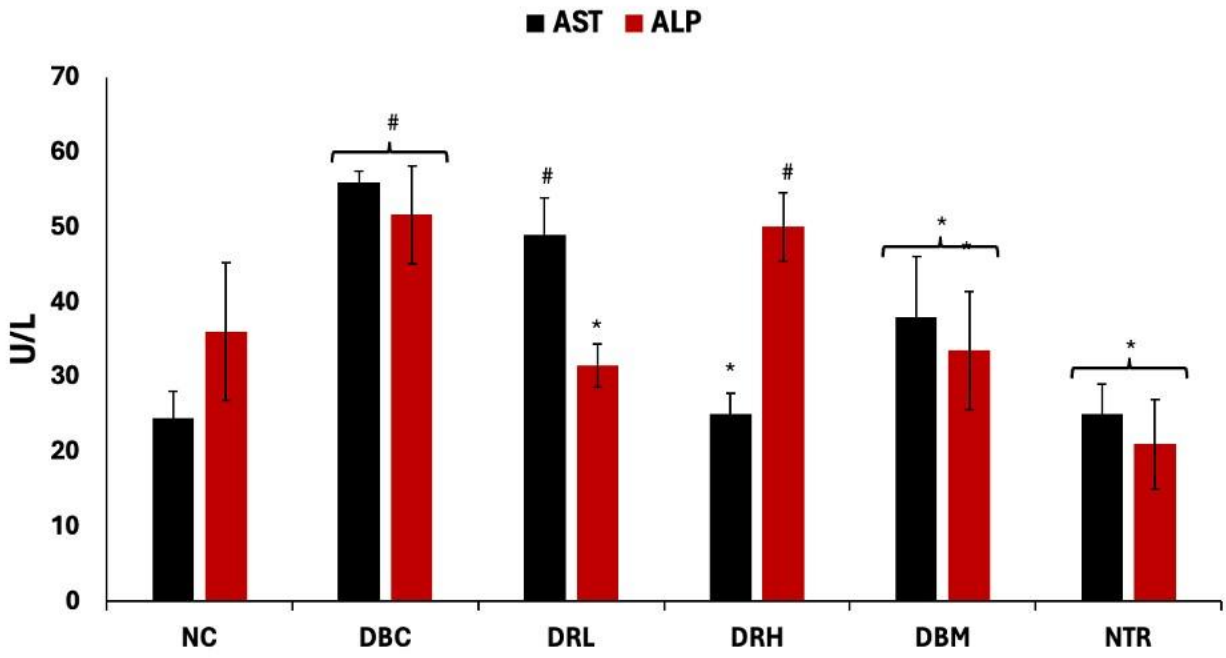


Figure 4.6: Effect of SPs on hepatic function markers levels in serum of T2D rats. Values = mean±SD; n=6. *Statistically significant ($p<0.05$) to DBC; #statistically ($p<0.05$) significant to NC).

GC-MS analysis revealed that hepatic metabolites of the NC group consist of urea, alphaketoglutaric acid, palmitoleic acid, pentadecanoic acid, linoleic acid, (9e)-9-octadecenoic acid, cisvaccenic acid, octadecanoic acid, arachidonic acid, 12-ketotridecanoic acid eicosapentaenoic acid, cholesteryl nonanoate, cholesterol and dihydrobrassicasterol as shown in Table 4.1. Induction of T2D altered these metabolites as depicted by the depleted levels of arachidonic acid, dihydrobrassicasterol, eicosapentaenoic acid cis-vaccenic acid. This was further depicted by the concomitant generation of nicotinamide, cholestadiene, 2-hexyloctanoic acid, linolenic acid, 24dehydrocholesterol, heptadecanoic acid, squalene, and docosahexanoic acid. Treatment with SPs at low dose restored the eicosapentaenoic acid level, while depleting T2D-generated nicotinamide, heptadecanoic acid, linolenic acid, docosahexanoic acid and 24-

dehydrocholesterol. At high dose, SPs depleted hepatic levels of pentadecanoic acid, linoleic acid, (9e)-9-octadecenoic acid, cisvaccenic acid, 12-ketotridecanoic acid and cholesteryl nonanoate. It also led to the depletion of T2D-generated nicotinamide, heptadecanoic acid and 24-dehydrocholesterol, while concomitantly generating glycerin, 3-methyldecanoic acid, pentadecanoic acid, cis-10-heptadecenoic acid, tetradecanoic acid, eicosanoic acid and 4,5-diamino-6-hydroxypyrimidine.

Table 4.1: Effect of SPs on hepatic metabolites of T2D rats.

Metabolites	NC	DBC	DRL	DRH	DBM	NTR
Urea	0.47±0.34	0.62±0.29	0.75±0.37	ND	ND	ND
alpha-Ketoglutaric acid	0.65±0.45	1.40±0.79	0.42±0.18	1.01±0.73	1.01±0.64	1.35±1.05
Palmitoleic acid	1.38±0.17	1.53±0.25	1.23±0.43	1.43±0.14	1.82±0.68	0.99±0.16
Pentadecanoic acid	12.14±4.12	8.02±1.81	5.40±1.12	ND	8.18±1.02	0.20±0.10
Linoleic acid	1.53±0.19	3.91±1.08	1.15±0.22	ND	5.19±1.14	4.59±1.47
(9E)-9-Octadecenoic acid	6.44±0.30	3.43±0.41	1.41±0.12	ND	3.90±0.38	ND
cis-Vaccenic acid	3.99±1.27	ND	ND	ND	ND	ND
Octadecanoic acid	4.61±0.05	4.21±0.37	5.01±0.49	10.06±3.00	5.60±2.41	8.01±1.81
Arachidonic acid	0.87±0.38	ND	1.37±0.41	0.95±0.43	4.01±0.07	5.48±1.22
12-Ketotridecanoic acid	0.68±0.08	0.75±0.12	1.05±0.38	ND	ND	1.18±0.24
Eicosapentaenoic acid	0.98±0.08	ND	0.84□0.17	ND	ND	0.87±0.04
Cholesteryl nonanoate	0.74±0.19	0.28±0.10	ND	ND	ND	0.41±0.06
Cholesterol	18.99±4.38	23.99±6.06	21.12±8.56	12.54±1.50	20.80±4.05	14.42±2.19
Dihydrobrassicasterol	2.04±0.36	ND	ND	ND	ND	ND
2-Hexyloctanoic acid	ND	2.99±0.18	1.92±0.19	0.29±0.13	ND	2.11±0.53
Nicotinamide	ND	0.51±0.08	ND	ND	ND	ND
Heptadecanoic acid	ND	0.29±0.12	ND	ND	ND	0.22±0.06

Linolenic acid	ND	0.57±0.10	ND	0.92±0.28	ND	ND
Docosahexanoic acid	ND	0.66±0.08	ND	1.13±0.16	ND	0.28±0.08
Squalene	ND	3.19±1.25	1.60±0.73	1.32±0.58	ND	ND
Cholestadiene	ND	0.88±0.38	0.50±0.17	0.74±0.10	0.96±0.02	0.70±0.03
24-Dehydrocholesterol	ND	0.23±0.01	ND	ND	ND	ND
17α-Hydroxypregnenolone	ND	ND	4.53±0.92	ND	ND	ND
Glycerin	ND	ND	ND	0.59±0.30	ND	ND
3-Methyldecanoic acid	ND	ND	ND	0.18±0.09	ND	ND
Pentadecanoic acid	ND	ND	ND	0.37±0.20	ND	ND
cis-10-Heptadecenoic acid	ND	ND	ND	0.23±0.10	ND	ND
Tetradecanoic acid	ND	ND	ND	0.41±0.19	ND	0.25±0.13
Eicosanoic acid	ND	ND	ND	0.36±0.13	ND	ND
4,5-Diamino-6hydroxypyrimidine	ND	ND	ND	0.41±0.23	ND	0.27±0.15
4-Amino-3-isothiazolecarboxylic acid	ND	ND	ND	ND	ND	0.26±0.14

Eicosenoic acid	ND	ND	ND	ND	ND	5.14±1.69

Values = mean±SD; n=6. ND = not detected.

As shown in Figure 4.7A, heatmap analysis revealed the distinct distribution of the hepatic metabolites among the treatment groups, with the DBL and DBC group portraying the most distinct changes as depicted by the positive value and color intensity. This was further portrayed by principal cluster analysis (PCA), which also depicted distinct changes in hepatic metabolites distribution between the SPCs treated groups and untreated diabetic group as shown by the score plots (Figure 4.7B). Biplot analysis of PCA components, PC1 (37.74%) and PC2 (24.76%) revealed strong relationships between treatment groups and the hepatic metabolites: 4,5-diamino6-hydroxypyrimidine, eicosenoic acid, pentadecanoic acid, urea, linolenic acid, docosahexanoic acid, arachidonic acid, tetradecanoic acid and 2-hexyloctanoic acid (Figure 4.7C).

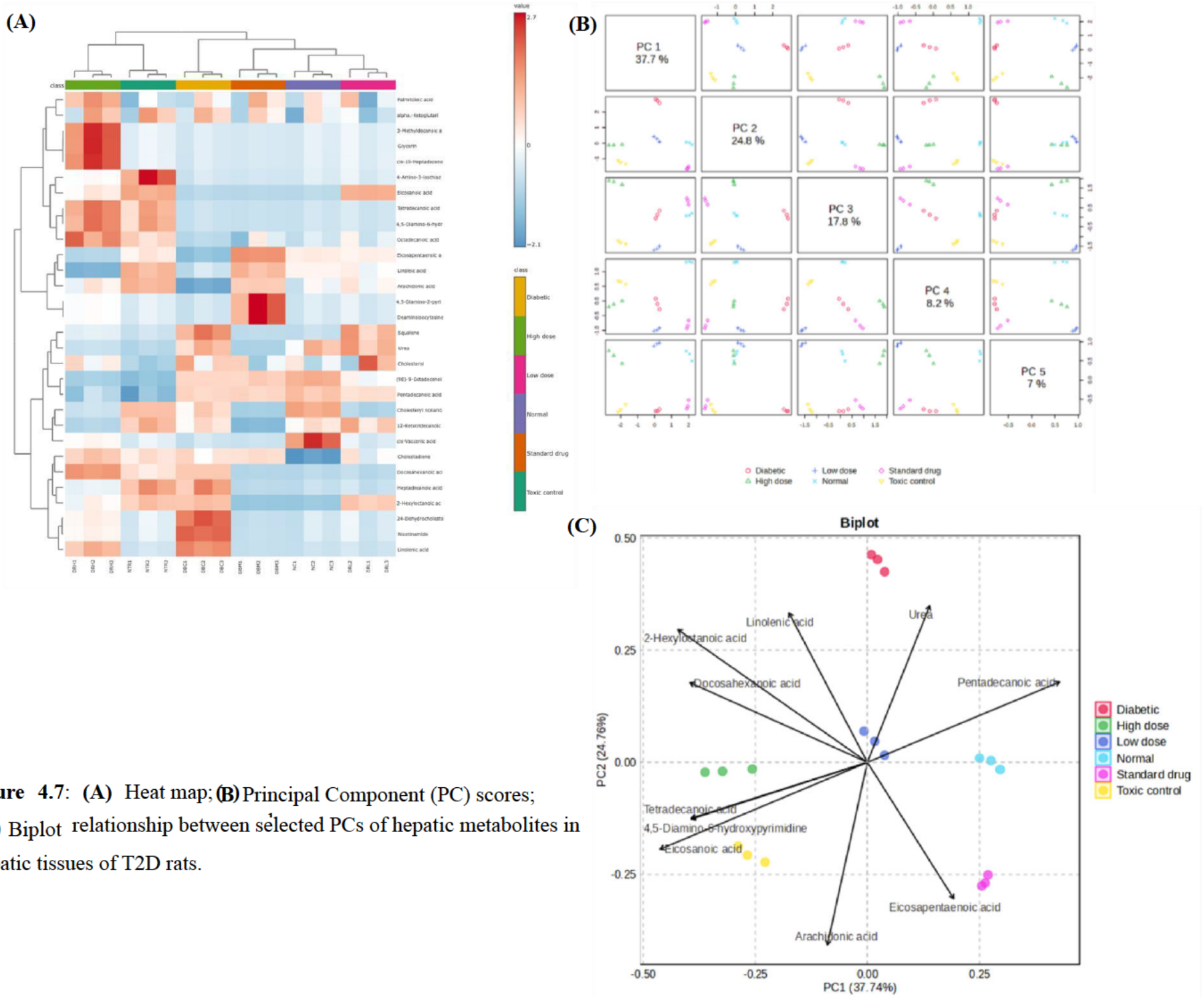
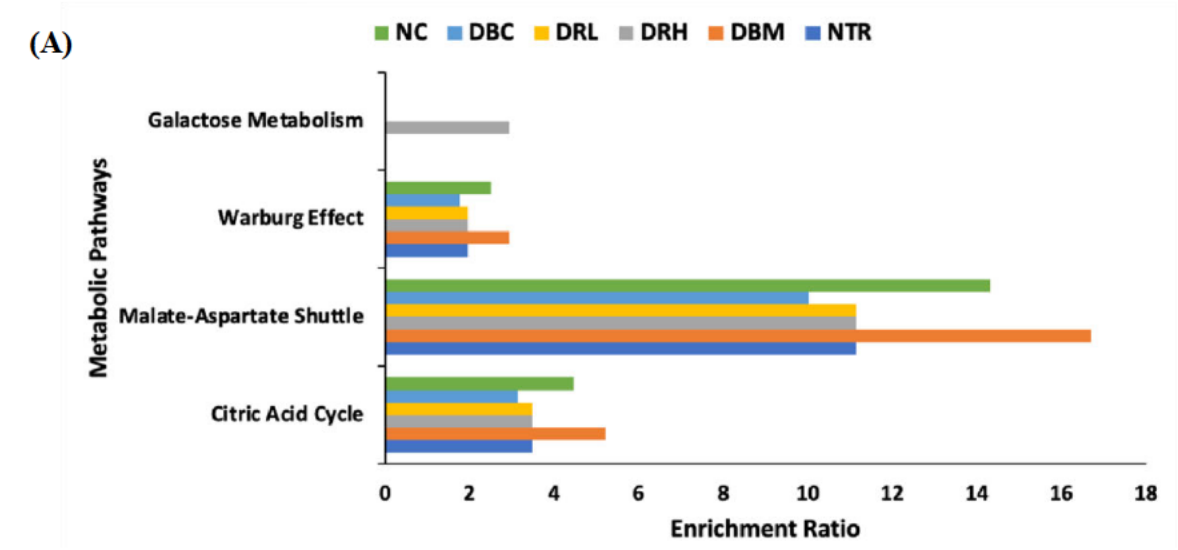


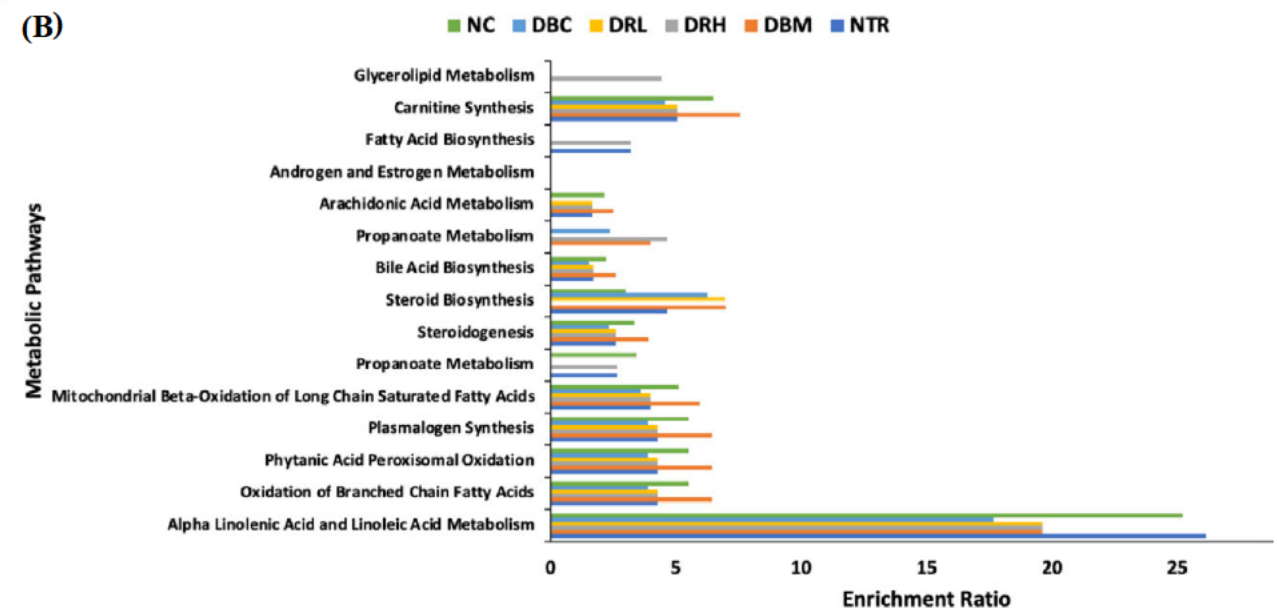
Figure 4.7: **(A)** Heat map; **(B)** Principal Component (PC) scores; **(C)** Biplot relationship between selected PCs of hepatic metabolites in hepatic tissues of T2D rats.

Pathway enrichment analysis of the identified hepatic metabolites revealed dysregulated glucose metabolism depicted by downregulated pathways for citric acid cycle, malate-aspartate shuttle, and Warburg effect (Figure 4.8A and Table 4.2). These pathways were upregulated following treatment with both doses of SPs. At high dose, treatment with SPs led to the activation of galactose metabolism.

Induction of T2D also led to dysregulated lipid metabolism as shown in Figure 4.8B and Table 4.2. This is depicted by downregulation of alpha linolenic acid and linoleic acid metabolism, oxidation of branched chain fatty acids, phytanic acid peroxisomal oxidation, plasmalogen synthesis, mitochondrial beta-oxidation of long chain saturated fatty acids, steroidogenesis, carnitine synthesis and bile acid biosynthesis, while activating propanoate metabolism. Following treatment with SPs, the pathways were upregulated, while concomitantly inactivating propanoate metabolism. Fatty acid biosynthesis and glycerolipid metabolism were activated following treatment with SPs at high dose.

Furthermore, induction of T2D led to dysregulated amino acid metabolism. Treatment with SPs at low dose inactivated d-arginine and d-ornithine metabolism, glucose-alanine cycle, tryptophan metabolism and tyrosine metabolism, but upregulated the other pathways. The pathways, except d-arginine and d-ornithine metabolism, were upregulated following treatment with SPs at high dose.


(A)
NC
DBC
DRL
DRH
DBM
NTR
Metabolic Pathways
Galactose Metabolism
Warburg Effect
Malate-Aspartate Shuttle
Citric Acid Cycle
0
2
4
6
8
10
12
14
16
18
Enrichment Ratio


(B)
NC
DBC
DRL
DRH
DBM
NTR
Metabolic Pathways
Glycerolipid Metabolism
Carnitine Synthesis
Fatty Acid Biosynthesis
Androgen and Estrogen Metabolism
Arachidonic Acid Metabolism
Propanoate Metabolism
Bile Acid Biosynthesis
Steroid Biosynthesis
Steroidogenesis
Propanoate Metabolism
Mitochondrial Beta-Oxidation of Long Chain Saturated Fatty Acids
Plasmalogen Synthesis
Phytanic Acid Peroxisomal Oxidation
Oxidation of Branched Chain Fatty Acids
Alpha Linolenic Acid and Linoleic Acid Metabolism
0
5
10
15
20
25
Enrichment Ratio

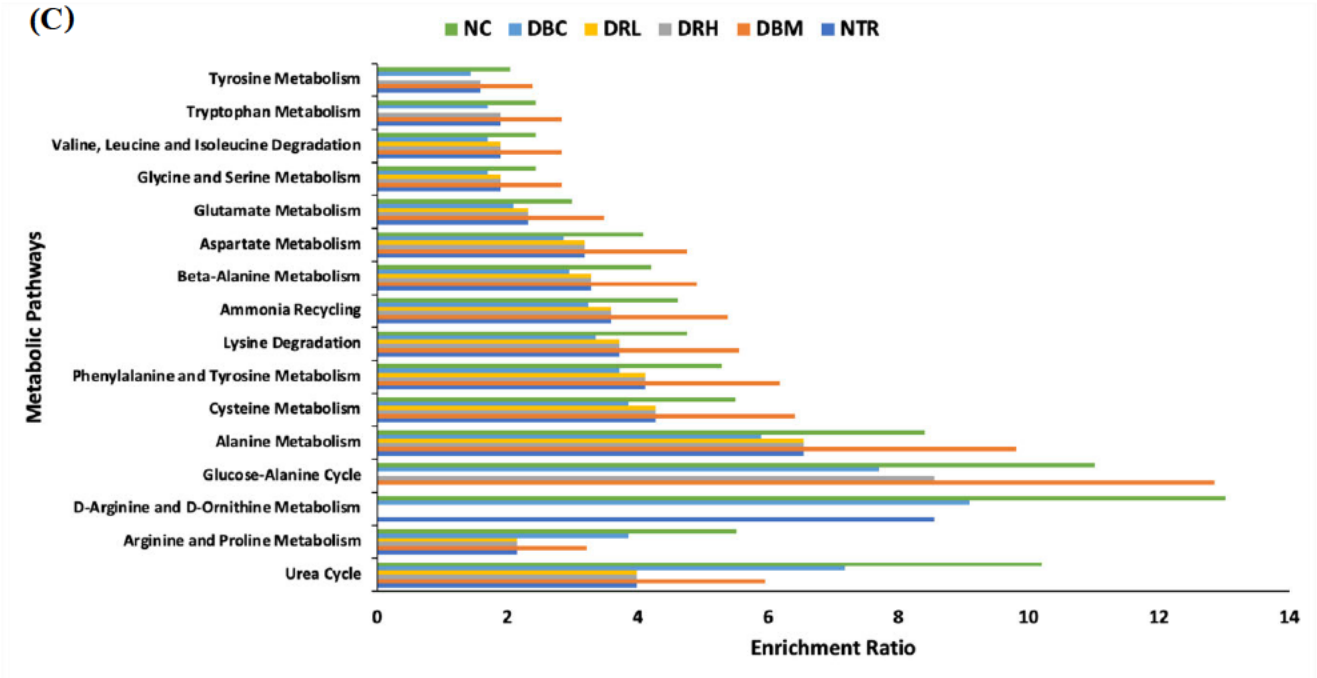

(C)
NC
DBC
DRL
DRH
DBM
NTR
Metabolic Pathways
Tyrosine Metabolism
Tryptophan Metabolism
Valine, Leucine and Isoleucine Degradation
Glycine and Serine Metabolism
Glutamate Metabolism
Aspartate Metabolism
Beta-Alanine Metabolism
Ammonia Recycling
Lysine Degradation
Phenylalanine and Tyrosine Metabolism
Cysteine Metabolism
Alanine Metabolism
Glucose-Alanine Cycle
D-Arginine and D-Ornithine Metabolism
Arginine and Proline Metabolism
Urea Cycle
0
2
4
6
8
10
12
14
Enrichment Ratio

Figure 4.8: Pathway enrichment of hepatic metabolites for (A) glucose, (B) amino acids, and (C) lipid metabolism in hepatic tissues of T2D rats.

Table 4.2: Pathway of identified hepatic metabolites of Type-2 Diabetic rats.

Metabolism	Pathways	NC	DBC	DRL	DRH	DBM	NTR
Glucose metabolism	Citric Acid Cycle	✓	↓	↑	↑	↑	↑
	Malate-Aspartate Shuttle	✓	↓	↑	↑	↑	↑
	Warburg Effect	✓	↓	↑	↑	↑	↑
	Galactose Metabolism	✗	✗	✗	✓	✗	✗
Lipid Metabolism	Alpha Linolenic Acid and Linoleic Acid Metabolism	✓	↓	↑	↑	↑	↑
	Oxidation of Branched Chain Fatty Acids	✓	↓	↑	↑	↑	↑
	Phytanic Acid Peroxisomal Oxidation	✓	↓	↑	↑	↑	↑
	Plasmalogen Synthesis	✓	↓	↑	↑	↑	↑
	Mitochondrial Beta-Oxidation of Long Chain Saturated Fatty Acids	✓	↓	↑	↑	↑	↑
	Steroidogenesis	✓	↓	↑	↑	↑	↓
	Steroid Biosynthesis	✓	↑	↓	✗	↑	↑
	Bile Acid Biosynthesis	✓	↓	↑	↑	↑	↓
	Propanoate Metabolism	✗	✓	✗	↓	✗	↓
	Arachidonic Acid Metabolism	✓	✗	↑	↑	↑	↓
	Fatty Acid Biosynthesis	✗	✗	✗	✓	✗	✗
	Carnitine Synthesis	✓	↓	↑	↑	↑	↑
	Glycerolipid Metabolism	✗	✗	✗	✓	✗	✗

Amino acid metabolism	Urea Cycle	✓	↓	↓	↓	↓	↓
	Arginine and Proline Metabolism	✓	↓	↓	↓	↓	↓
	D-Arginine and D-Ornithine Metabolism	✓	↓	✗	✗	✗	↓
	Glucose-Alanine Cycle	✓	↓	✗	↑	↑	✗
	Alanine Metabolism	✓	↓	↑	↑	↑	↑
	Cysteine Metabolism	✓	↓	↑	↑	↑	↑
	Phenylalanine and Tyrosine Metabolism	✓	↓	↑	↑	↑	↑
	Lysine Degradation	✓	↓	↑	↑	↑	↑
	Ammonia Recycling	✓	↓	↑	↑	↑	↑
	Beta-Alanine Metabolism	✓	↓	↑	↑	↑	↑
	Aspartate Metabolism	✓	↓	↑	↑	↑	↑
	Glutamate Metabolism	✓	↓	↑	↑	↑	↑
	Glycine and Serine Metabolism	✓	↓	↑	↑	↑	↑
	Valine, Leucine and Isoleucine Degradation	✓	↓	↑	↑	↑	↑
	Tryptophan Metabolism	✓	↓	✗	↑	↑	↑
	Tyrosine Metabolism	✓	↓	✗	↑	↑	↑

✓ = present; ↓ = downregulated; ↑ = upregulated; ✗ = inactivated; — = no change

4.4 Discussion

The increasing prevalence of diabetic hepatopathy is a major concern to health practitioners, as it is among the main contributors to T2D mediated mortality (Islam et al. 2020; Mallet et al. 2022; Mobasheri et al. 2023; Tolman et al. 2007). This have led to the continuous search of affordable therapy, with a paradigm shift towards natural products. Among these natural products, are sulphated polysaccharides from seaweeds. In the present study, we investigated the effect of SPs from *G. gracilis* on diabetic hepatopathy in male rats with T2D.

There's a known connection between glucose production in the liver and diabetic hepatopathy, which contributes to hyperglycemia in diabetes (Barroso et al. 2024; Zhang et al. 2019). Glycogen breakdown, hyperglycemia, has also been found to occur continuously in diabetic hepatopathy, especially in T2D (Jiang et al. 2020; Rines et al. 2016). The elevated activities of fructose-1,6-biphosphatase, glucose 6-phosphatase and glycogen phosphorylase in the DBC group (Figure 4.1), indicate gluconeogenesis and glycogenolysis on induction of T2D. Glycogen phosphorylase plays a crucial role in glycogenolysis, catalysing the release of glucose-1-phosphate from glycogen. The glucose-1-phosphate is then converted to glucose-6-phosphate. The elevated activities of these enzymes suggest an arrest of glycolysis and correlates with the dysregulation of glucose metabolizing pathways vis-à-vis citric acid cycle, malate-aspartate shuttle and Warburg effect (Tables 4.2 and 3.3; and Figure 4.8A). This leads to suppressed hepatic ATP levels which has been reported in diabetic hepatopathy (Koliaki and Roden 2013; Schmid et al. 2011). The suppression of these enzyme activities, as well as upregulation of glucose metabolism pathways in SPs-treated diabetic rats, indicate an arrest of gluconeogenesis and glycogenolysis. Thus, suggesting activation of glucose oxidation (glycolysis and citric acid cycle) and improved hepatic ATP production. This corresponds with previous reports on the inhibition of gluconeogenesis and glycogenolysis as

therapeutic strategy in the management of diabetic hepatopathy (Rines et al. 2016). Therefore, portraying the therapeutic potential of SPs in improving hepatic glucose homeostasis in T2D.

Continuous gluconeogenesis and glycogenolysis in T2D, have been implicated in increased cellular glucose level. The excess glucose is often channelled into other metabolic pathways to produce toxic metabolites which are mediators of hyperglycemia toxicity. Excess glucose may undergo enolization to generate superoxide anion ($O_2^{\cdot -}$) and reactive ketoaldehydes radicals, which causes a shift in the cells' oxidant-antioxidant balance system towards the former, leading to oxidative stress (Snezhkina et al. 2019). The generated $O_2^{\cdot -}$ is further reduced by SOD to hydrogen peroxide (H_2O_2). If not reduced by glutathione peroxidase and catalase to water (H_2O) and O_2, H_2O_2 will be further broken down to hydroxyl radicals ($OH\cdot$) (Meyerstein 2021). Our findings revealed significant reduction in hepatic catalase and SOS activities in the DBC group after induction of T2D (Figures 4.2A and 4.2B). These reduced enzyme activities indicate an occurrence of hepatic oxidative stress, following the induction of T2D. This corroborates previous studies linking oxidative stress with the pathophysiology of diabetic hepatopathy (Caturano et al. 2023; Oguntibeju 2019). Therefore, the elevated activities of these enzymes, following treatment with SPs, suggest an antioxidative effect which has been reported a therapeutic strategy in the management of diabetic hepatopathy (Mobasheri et al. 2023; Mohamed et al. 2009).

Excess glucose can also be diverted to the polyol pathway, where it is converted into sorbitol by aldose reductase. Sorbitol is subsequently converted to fructose in a reaction catalysed by sorbitol dehydrogenase (Erukainure and Chukwuma 2024). In the initial stage of the pathway, aldose reductase disrupts the oxidation of NADH to NAD+, resulting in an alteration of glutathione metabolism and subsequently oxidative stress (Brownlee 2005). This is depicted in the present study by the suppressed hepatic GSH level in untreated diabetic rats (DBC) (Figure 4A). This is portrayed by the reduced hepatic activities of glutathione reductase, glutathione peroxidase, and

glutathione s-transferase (GST) (Figures 4.3B – D). Glutathione redssuctase catalyzes the reduction of oxidized glutathione (GSSG) to its reduced form, GSH, whereas glutathione peroxidase catalyzes the oxidation of GSH to GSSG (Couto et al. 2016; Erukainure and Chukwuma 2024). These decreased enzymes activities portray perturbed GSH homeostasis, which also indicates an occurrence of hepatic oxidative stress. The decreased GST activity further indicate elevated ROS levels (Szeligowska et al. 2022; Zhang et al. 2021). The increased enzymes activities and GSH level in the SPs-treated diabetic rats, indicate improved glutathione metabolism. This also corroborates the improved antioxidative activities.

In the absence of sufficient catalase and glutathione peroxidase activity, $OH\cdot$ from the degradation of H_2O_2 target the cell lipid membrane, initiating a lipid peroxidation cascade which produces malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) (Ighodaro and Akinloye 2018; Jena et al. 2023). The elevated hepatic MDA level in the untreated diabetic rats (Figure 4.4), indicates lipid peroxidation on induction of T2D. Furthermore, observed hepatic oxidative stress correlates with the perturbed antioxidant enzyme activities and glutathione metabolism. Lipid peroxidation has been implicated in the pathophysiology of diabetic hepatopathy, and a therapeutic target in the disease management (de Souza Bastos et al. 2016; Shabalala et al. 2022). Thus, the decreased MDA levels following treatment with SPs, indicates an ameliorative effect on lipid peroxidation and preventive mechanism against oxidative damage to hepatic tissues. This also corroborates SPs antioxidative activities in diabetic hepatopathy.

Elevated acetylcholinesterase activity in hepatic tissues has been linked to low-grade systemic inflammation in diabetic hepatopathy (Das 2012; Mahmood et al. 2021). The enzyme hydrolyzes acetylcholine to choline and acetate, thus, indicating that the elevated activity of the enzyme in hepatic tissues of untreated diabetic rats (Figure 4.5), suggests elevated cellular levels of choline and acetate which may contribute to inflammation. This corroborates previous reports on elevated

acetylcholinesterase activity in diabetic hepatic tissues (Erukainure et al. 2021). The reduced enzyme activity following treatment with SPs, indicates improved cellular levels of acetylcholine, and suggests an antiinflammatory effect. Acetylcholine binds to the α-7 subunits of nicotinic acetylcholine receptors, which activates the cholinergic anti-inflammatory pathway and suppresses the release of cytokines (Bondok et al. 2013).

Dysregulated hepatic amino acid metabolism has been reported as part of the pathophysiology of diabetic hepatopathy, with insulin resistance being the main trigger (Cuomo et al. 2022; Wiklund et al. 2018). These dysregulations are often associated with increased serum levels of transaminases (Judi et al. 2010; Yazdi et al. 2019). This is depicted in the present study by the dysregulated amino acid metabolic pathways, and concomitant elevated serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) in the untreated diabetic rats (Figures 4.6 and 4.8C, and Table 4.2). These dysregulated pathways can be attributed to the depleted hepatic urea and alpha-ketoglutaric acid levels, with AST and ALT activities playing influential roles. ALT converts alanine to pyruvate and glutamate, by transferring an amino group from alanine to αketoglutarate, a crucial step in amino acid metabolism (reaction 1). The reversal of these reaction is regarded as the glucose-alanine cycle, which drives gluconeogenesis. The increased serum level of ALT indicates depleted hepatic availability of the enzyme for transamination activity. This is further portrayed by the reduced hepatic level of α-ketoglutarate (Table 4.1) and dysregulated glucose-alanine cycle, alanine metabolism, beta-alanine metabolism and glutamate metabolism pathways (Figure 4.8C and Table 2).

$$\text{Alanine} + \alpha\text{-Ketoglutarate} \rightleftharpoons \text{Pyruvate} + \text{Glutamate} \qquad (1)$$

AST converts aspartate to glutamate and oxaloacetate, by catalyzing the transfer of amino group from aspartate to alpha-ketoglutarate (reaction 2).

$$\text{Aspartate} + \alpha\text{-Ketoglutarate} \leftrightarrow \text{Oxaloacetate} + \text{Glutamate} \quad (2)$$

The generated glutamate from both reactions is converted to ammonia (reaction 3), which enters the urea cycle. This corresponds with the dysregulated urea cycle, ammonia recycling, aspartate metabolism, and glutamate metabolism pathways.

$$\text{Glutamate} + NAD^+ + H_2O \rightarrow \alpha\text{−Ketoglutarate} + NH_3 + NADH + H^+ \quad (3)$$

The upregulation of these pathways and alpha-ketoglutarate concentration, as well as concomitant decreased serum levels of AST and ALT in the SPs-treated diabetic rats, indicates improved hepatic amino acid metabolism. Alterations in hepatic lipid metabolism and its metabolites, characterized by fat accumulation, distorted fatty acid oxidation and hepatic steroid metabolism, leading to hepatic lipotoxicity is an hallmark of diabetic hepatopathy (Gluchowski et al. 2017; Mendez-Sanchez et al. 2018). This is demonstrated in the present study by the altered free fatty acids (FFAs) levels, and concomitant generation of additional FFAs, as well as their disparity in hepatic tissues of untreated diabetic rats and other diabetic groups in the study (Table 4.1 and Figures 4.7A – 4.6C). This indicates an occurrence of hepatic fat accumulation and lipotoxicity on induction of T2D. This is further corroborated by the dysregulated hepatic lipid metabolic pathways, particularly oxidation of branched chain fatty acids, phytanic acid peroxisomal oxidation, and mitochondrial beta-oxidation of long chain saturated fatty acids pathways (Figure 4.8 B and Table 4.2). The dysregulated steroidogenesis, steroid biosynthesis and bile acid biosynthesis pathways, may be attributed to alterations in hepatic cholesterol level and other cholesteric metabolites. These distortions corroborate previous reports on dysregulated lipid metabolic pathways and metabolites in hepatic tissues of diabetic rats (Erukainure et al. 2021). The improved hepatic FFAs levels and constituents, with concomitant upregulation of lipid metabolic pathways following treatment with SPs, indicate suppressed hepatic lipid accumulation and improved metabolism. Thus, suggesting an anti-lipotoxic effect on diabetic hepatopathy.

4.5 Conclusion

Diabetic hepatopathy is a major complication of diabetes which involves a complex interplay of distorted metabolisms. It encompasses hyperglycemia, oxidative stress, inflammation, exacerbated fat accumulation, perturbed hepatic steroidogenesis, and dysregulated amino acid metabolism, subsequently leading to hepatic damage and dysfunction. *G. gracilis* SPs conferred a therapeutic effect against diabetic hepatopathy in T2D by improving glucose and amino acid metabolisms, with concomitant anti-lipotoxic, antioxidative and antiinflammatory effects. Further studies are required to translate these therapeutic potentials in clinical trials.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported by a grant from Technology Innovation Agency (TIA), Durban, South Africa (Grant no. SABDI 16/1072), and Incentives for Rated Researchers by the National Research Foundation (NRF).

Data availability statement

The data in support this research findings are presented in the article.

4.6. Reference

ADA (2015) 2. Classification and diagnosis of diabetes. Diabetes care 38(Supplement_1):S8-S16

Adams SH (2011) Emerging perspectives on essential amino acid metabolism in obesity and the insulin-resistant state. Advances in nutrition 2(6):445-456

Adeghate E, D'Souza CM, Saeed Z, et al. (2021) Nociceptin increases antioxidant expression in the kidney, liver and brain of diabetic rats. Biology 10(7):621

Adeva-Andany MM, Pérez-Felpete N, Fernández-Fernández C, Donapetry-García C, Pazos-García C (2016) Liver glucose metabolism in humans. Bioscience reports 36(6):e00416

Aebi H (1984) Catalase in vitro. Methods in Enzymology 105:121-126

Ahmed N (2005) Advanced glycation endproducts—role in pathology of diabetic complications. Diabetes research and clinical practice 67(1):3-21

Åkerblom HK, Vaarala O, Hyöty H, Ilonen J, Knip M (2002) Environmental factors in the etiology of type 1 diabetes. American Journal of Medical Genetics Part A 115(1):18-29

Al-Dalali S, Li C, Xu B (2022) Effect of frozen storage on the lipid oxidation, protein oxidation, and flavor profile of marinated raw beef meat. Food Chemistry 376:131881

Antar SA, Ashour NA, Sharaky M, et al. (2023) Diabetes mellitus: Classification, mediators, and complications; A gate to identify potential targets for the development of new effective treatments. Biomedicine & Pharmacotherapy 168:115734

Aronson D (2008) Hyperglycemia and the pathobiology of diabetic complications Cardiovascular Diabetology: Clinical, Metabolic and Inflammatory Facets. vol 45. Karger Publishers, p 116

Azanha A, Faria J (2005) Use of mathematical models for estimating the shelf-life of cornflakes in flexible packaging. Packaging Technology and Science: An International Journal 18(4):171178

Bai Z, Huang X, Wu G, et al. (2023) Hepatic metabolism-related effects of polysaccharides from red kidney bean and small black soybean on type 2 diabetes. Food Chemistry 403:134334

Bajaj PR, Bhunia K, Kleiner L, et al. (2017) Improving functional properties of pea protein isolate for microencapsulation of flaxseed oil. Journal of Microencapsulation 34(2):218-230

Balogun F, Ashafa A (2017) Aqueous root extracts of Dicoma anomala (Sond.) extenuates postprandial hyperglycaemia in vitro and its modulation on the activities of carbohydratemetabolizing enzymes in streptozotocin-induced diabetic Wistar rats. South African journal of botany 112:102-111

Barb D, Repetto EM, Stokes ME, Shankar SS, Cusi K (2021) Type 2 diabetes mellitus increases the risk of hepatic fibrosis in individuals with obesity and nonalcoholic fatty liver disease. Obesity 29(11):1950-1960

Barroso E, Jurado-Aguilar J, Wahli W, Palomer X, Vázquez-Carrera M (2024) Increased hepatic gluconeogenesis and type 2 diabetes mellitus. Trends in Endocrinology & Metabolism

Bauman P, Smith T, Bray T (1988) The effect of dietary protein and sulfur amino acids on hepatic glutathione concentration and glutathione-dependent enzyme activities in the rat. Canadian journal of physiology and pharmacology 66(8):1048-1052

Bhadja P, Tan C-Y, Ouyang J-M, Yu K (2016) Repair effect of seaweed polysaccharides with different contents of sulfate group and molecular weights on damaged HK-2 cells. Polymers 8(5):188

Bondok RS, Ahmed MA, Soliman NBE, et al. (2013) The effect of cholinesterase inhibition on liver dysfunction in experimental acute liver failure. The Egyptian Journal of Critical Care Medicine 1(2):51-59

Borazjani NJ, Tabarsa M, You S, Rezaei M (2018) Purification, molecular properties, structural characterization, and immunomodulatory activities of water soluble polysaccharides from Sargassum angustifolium. International journal of biological macromolecules 109:793-802

Bösenberg LH, van Zyl DG (2008) The mechanism of action of oral antidiabetic drugs: a review of recent literature. Journal of Endocrinology, Metabolism and Diabetes of South Africa 13(3):80-88

Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414(6865):813

Brownlee M (2005) The pathobiology of diabetic complications: a unifying mechanism. Diabetes 54(6):1615-1625

Bulum T, Kolarić B, Duvnjak L, Duvnjak M (2011) Nonalcoholic fatty liver disease markers are associated with insulin resistance in type 1 diabetes. Digestive diseases and sciences 56:3655-3663

Bunner AE, Chandrasekera PC, Barnard ND (2014) Knockout mouse models of insulin signaling: Relevance past and future. World journal of diabetes 5(2):146

Burrack AL, Martinov T, Fife BT (2017) T cell-mediated beta cell destruction: autoimmunity and alloimmunity in the context of type 1 diabetes. Frontiers in endocrinology 8:343

Butalia S, Kaplan GG, Khokhar B, Rabi DM (2016) Environmental risk factors and type 1 diabetes: past, present, and future. Canadian journal of diabetes 40(6):586-593

Çalışkan Koç G, Özkan Karabacak A, Süfer Ö, et al. (2025) Thawing frozen foods: A comparative review of traditional and innovative methods. Comprehensive Reviews in Food Science and Food Safety 24(2):e70136

Calligaris S, Manzocco L, Anese M, Nicoli MC (2019) Accelerated shelf life testing Food quality and shelf life. Elsevier, p 359-392

Campbell I (2007) Oral antidiabetic drugs: their properties and recommended use. Prescriber 18(6):56-74

Carpentier AC (2021) 100th anniversary of the discovery of insulin perspective: insulin and adipose tissue fatty acid metabolism. American Journal of Physiology-Endocrinology and Metabolism 320(4):E653-E670

Catalfamo DL, Britten TM, Storch DI, Calderon NL, Sorenson HL, Wallet SM (2013) Hyperglycemia induced and intrinsic alterations in type 2 diabetes-derived osteoclast function. Oral diseases 19(3):303-312

Caturano A, D'Angelo M, Mormone A, et al. (2023) Oxidative stress in type 2 diabetes: impacts from pathogenesis to lifestyle modifications. Current Issues in Molecular Biology 45(8):6651-6666

Cernea S, Dobreanu M (2013) Diabetes and beta cell function: from mechanisms to evaluation and clinical implications. Biochemia medica 23(3):266-280

Chan CXa, Khan AA, Choi JH, et al. (2013) Technology platform development for targeted plasma metabolites in human heart failure. Clinical Proteomics 10(1):7

Chang S-C, Yang W-CV (2016) Hyperglycemia, tumorigenesis, and chronic inflammation. Critical reviews in oncology/hematology 108:146-153

Chen K, Zhao J, Shi X, Abdul Q, Jiang Z (2019a) Characterization and antioxidant activity of products derived from xylose–bovine casein hydrolysate Maillard reaction: Impact of reaction time. Foods 8(7):242

Chen W, Xu W, Junejo SA, et al. (2024) Analytical methods for starch retrogradation assessment. Food Biomacromolecules 1(2):107-126

Chen X, Jin J, Tang J, et al. (2011) Extraction, purification, characterization and hypoglycemic activity of a polysaccharide isolated from the root of Ophiopogon japonicus. Carbohydrate Polymers 83(2):749-754

Chen Y, Liu D, Wang D, et al. (2019b) Hypoglycemic activity and gut microbiota regulation of a novel polysaccharide from Grifola frondosa in type 2 diabetic mice. Food and Chemical Toxicology 126:295-302

Chiacchiaretta P, Tumini S, Mascitelli A, et al. (2024) The Impact of Atmospheric Temperature Variations on Glycaemic Patterns in Children and Young Adults with Type 1 Diabetes. Climate 12(8):121

Chikezie PC, Ojiako OA, Ogbuji AC (2015) Oxidative stress in diabetes mellitus. Integrative Obesity and Diabetes 9(3):92-109

Chiniwala N, Jabbour S (2011) Management of diabetes mellitus in the elderly. Current Opinion in Endocrinology, Diabetes and Obesity 18(2):148-152

Chowdhury P, Soulsby M (2002) Lipid peroxidation in rat brain is increased by simulated weightlessness and decreased by a soy-protein diet. Annals of Clinical & Laboratory Science 32(2):188-192

Ciancia M, Fernández PV, Leliaert F (2020) Diversity of sulfated polysaccharides from cell walls of coenocytic green algae and their structural relationships in view of green algal evolution. Frontiers in plant science 11:554585

Cornblath M, Randle P, Parmeggiani A, Morgan H (1963) Regulation of glycogenolysis in muscle effects of glucagon and anoxia on lactate production, glycogen content, and phosphorylase activity in the perfused isolated rat heart. Journal of Biological Chemistry 238(5):1592-1597

Couto N, Wood J, Barber J (2016) The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. Free radical biology and medicine 95:27-42

Cucca F, Dudbridge F, Loddo M, et al. (2001) The HLA-DPB1–associated component of the IDDM1 and its relationship to the major loci HLA-DQB1,-DQA1, and-DRB1. Diabetes 50(5):12001205

Cuomo P, Capparelli R, Iannelli A, Iannelli D (2022) Role of branched-chain amino acid metabolism in type 2 diabetes, obesity, cardiovascular disease and non-alcoholic fatty liver disease. International journal of molecular sciences 23(8):4325

Das UN (2012) Acetylcholinesterase and butyrylcholinesterase as markers of low-grade systemic inflammation. Annals of hepatology 11(3):409-411

Daub CD, Mabate B, Malgas S, Pletschke BI (2020) Fucoidan from Ecklonia maxima is a powerful inhibitor of the diabetes-related enzyme, α-glucosidase. International journal of biological macromolecules 151:412-420

Dawi J, Misakyan Y, Affa S, et al. (2024) Oxidative Stress, Glutathione Insufficiency, and Inflammatory Pathways in Type 2 Diabetes Mellitus: Implications for Therapeutic Interventions. Biomedicines 13(1):18 de Jesus Raposo MF, De Morais AMB, De Morais RMSC (2015) Marine polysaccharides from algae with potential biomedical applications. Marine drugs 13(5):2967-3028

de la Monte SM, Wands JR (2008) Alzheimer's disease is type 3 diabetes—evidence reviewed. Journal of Diabetes Science and Technology 2(6):1101-1113

De Marco R, Locatelli F, Zoppini G, Verlato G, Bonora E, Muggeo M (1999) Cause-specific mortality in type 2 diabetes. The Verona Diabetes Study. Diabetes care 22(5):756-761

de Souza Bastos A, Graves DT, de Melo Loureiro AP, et al. (2016) Diabetes and increased lipid peroxidation are associated with systemic inflammation even in well-controlled patients. Journal of Diabetes and its Complications 30(8):1593-1599

Defronzo RA (1997) Pathogenesis of type2 daiabetes: metabolic and molecular implications for identhfying diabetes genes. Diabetes Reviews 5:177-269

DeFronzo RA (2009) From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 58(4):773-795

DeFronzo RA (2018) Pathogenesis of Type 2 Diabetes Mellitus. In: Bonora E, DeFronzo RA (eds) Diabetes Epidemiology, Genetics, Pathogenesis, Diagnosis, Prevention, and Treatment. Springer International Publishing, Cham, p 181-253

DeFronzo RA, Ferrannini E, Groop L, et al. (2015) Type 2 diabetes mellitus. Nature Reviews Disease Primers 1:15019

Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA (2011) Insulin effects in muscle and adipose tissue. Diabetes research and clinical practice 93:S52-S59

Ding Y, Wang S, Lu J (2023) Unlocking the potential: amino acids' role in predicting and exploring therapeutic avenues for type 2 diabetes mellitus. Metabolites 13(9):1017

Diyorbek I (2025) Pathogenesis of chronic complications of type 2 diabetes mellitus and ways of their correction. Web of Medicine: Journal of Medicine, Practice and Nursing 3(2):15-17

Donmez D, Pinho L, Patel B, Desam P, Campanella OH (2021) Characterization of starch–water interactions and their effects on two key functional properties: Starch gelatinization and retrogradation. Current Opinion in Food Science 39:103-109

Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M (2001) Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. Journal of Clinical Investigation 108(9):1341-1348

DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Analytical chemistry 28(3):350-356

Eguchi N, Vaziri ND, Dafoe DC, Ichii H (2021) The role of oxidative stress in pancreatic β cell dysfunction in diabetes. International journal of molecular sciences 22(4):1509

El-Mir M-Y, Nogueira V, Fontaine E, Avéret N, Rigoulet M, Leverve X (2000) Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. Journal of Biological Chemistry 275(1):223-228

Ellman GL (1959) Tissue sulfhydryl groups. Archives of biochemistry and biophysics 82(1):70-77

Ellman GL, Courtney KD, Andres Jr V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology 7(2):88-95

Erkmen O, Bozoglu TF (2016) Food preservation by low temperatures. Food Microbiology: Principles into Practice John Wiley and Sons, Ltd, Chichester, UK

Erukainure OL, Chukwuma CI (2024) African walnut (Plukenetia conophora) oil promotes glucose uptake while improving energy metabolism and steroidogenesis and maintaining surface architecture in rat testes. Frontiers in Nutrition 11:1505453

Erukainure OL, Hafizur R, Kabir N, et al. (2018) Suppressive Effects of Clerodendrum volubile P Beauv.[Labiatae] Methanolic Extract and Its Fractions on Type 2 Diabetes and Its Complications. Frontiers in Pharmacology 9:8 doi:10.3389/fphar.2018.00008

Erukainure OL, Mopuri R, Oyebode OA, Koorbanally NA, Islam MS (2017) Dacryodes edulis enhances antioxidant activities, suppresses DNA fragmentation in oxidative pancreatic and hepatic injuries; and inhibits carbohydrate digestive enzymes linked to type 2 diabetes. Biomedicine & pharmacotherapy 96:37-47

Erukainure OL, Oyebode OA, Chuturgoon AA, et al. (2024) Potential molecular mechanisms underlying the ameliorative effect of Cola nitida (Vent.) Schott & Endl. on insulin resistance in rat skeletal muscles. Journal of Ethnopharmacology 319:117249

Erukainure OL, Oyebode OA, Ijomone OM, Chukwuma CI, Koorbanally NA, Islam MS (2019) Raffia palm (Raphia hookeri G. Mann & H. Wendl) wine modulates glucose homeostasis by enhancing insulin secretion and inhibiting redox imbalance in a rat model of diabetes induced by high fructose diet and streptozotocin. Journal of ethnopharmacology 237:159170

Erukainure OL, Salau VF, Bharuth V, Koorbanally NA, Islam MS (2020) Hyperglycemia alters lipid metabolism and ultrastructural morphology of cerebellum in brains of diabetic rats: Therapeutic potential of raffia palm (Raphia hookeri G. Mann & H. Wendl) wine. Neurochemistry International 140:104849

Erukainure OL, Sanni O, Salau VF, Koorbanally NA, Islam MS (2021) Cola nitida (kola nuts) attenuates hepatic injury in type 2 diabetes by improving antioxidant and cholinergic dysfunctions and dysregulated lipid metabolism. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders) 21(4):688-699

Feng W, Ma S, Wang X (2020) Recent advances in quality deterioration and improvement of starch in frozen dough. Grain & Oil Science and Technology 3(4):154-163

Fernandes PA, Coimbra MA (2023) The antioxidant activity of polysaccharides: A structurefunction relationship overview. Carbohydrate Polymers 314:120965

Gancedo JM, Gancedo C (1971) Fructose-1, 6-diphosphatase, phosphofructokinase and glucose6-phosphate dehydrogenase from fermenting and non fermenting yeasts. Archiv für Mikrobiologie 76(2):132-138

Ganeshpurkar A, Kohli S, Rai G (2014) Antidiabetic potential of polysaccharides from the white oyster culinary-medicinal mushroom Pleurotus florida (higher Basidiomycetes). International Journal of Medicinal Mushrooms 16(3)

Garcia-Compean D, Jaquez-Quintana JO, Gonzalez-Gonzalez JA, Maldonado-Garza H (2009) Liver cirrhosis and diabetes: risk factors, pathophysiology, clinical implications and management. World journal of gastroenterology: WJG 15(3):280

Geng L, Liu K, Zhang H (2023) Lipid oxidation in foods and its implications on proteins. Frontiers in Nutrition 10:1192199

Geng T, Huang T (2020) Gene-environment interactions and type 2 diabetes. Asia Pacific Journal of Clinical Nutrition 29(2):220-226

Giordano S, Martocchia A, Toussan L, et al. (2014) Diagnosis of hepatic glycogenosis in poorly controlled type 1 diabetes mellitus. World journal of diabetes 5(6):882

Giri B, Dey S, Das T, Sarkar M, Banerjee J, Dash SK (2018) Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection,

cancer progression and other pathophysiological consequences: An update on glucose toxicity. Biomedicine & pharmacotherapy 107:306-328

Giwa AM, Ahmed R, Omidian Z, et al. (2020) Current understandings of the pathogenesis of type 1 diabetes: Genetics to environment. World journal of diabetes 11(1):13

Gluchowski NL, Becuwe M, Walther TC, Farese Jr RV (2017) Lipid droplets and liver disease: from basic biology to clinical implications. Nature reviews Gastroenterology & hepatology 14(6):343-355

Goldin A, Beckman JA, Schmidt AM, Creager MA (2006) Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation 114(6):597-605

González P, Lozano P, Ros G, Solano F (2023) Hyperglycemia and oxidative stress: An integral, updated and critical overview of their metabolic interconnections. International Journal of Molecular Sciences 24(11):9352

Gupta RK, Pipliya S, Karunanithi S, et al. (2024) Migration of Chemical Compounds from Packaging Materials into Packaged Foods: Interaction, Mechanism, Assessment, and Regulations. Foods 13(19):3125

Gustafson B, Hedjazifar S, Gogg S, Hammarstedt A, Smith U (2015) Insulin resistance and impaired adipogenesis. Trends in Endocrinology & Metabolism 26(4):193-200

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. Journal of biological Chemistry 249(22):7130-7139

Hakonarson H, Grant SF, Bradfield JP, et al. (2007) A genome-wide association study identifies KIAA0350 as a type 1 diabetes gene. Nature 448(7153):591-594

Han H-S, Kang G, Kim JS, Choi BH, Koo S-H (2016) Regulation of glucose metabolism from a livercentric perspective. Experimental & molecular medicine 48(3):e218-e218

Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW (2016) Non-alcoholic fatty liver disease and diabetes. Metabolism 65(8):1096-1108

Heaton JW, Marangoni AG (1996) Chlorophyll degradation in processed foods and senescent plant tissues. Trends in Food Science & Technology 7(1):8-15

Hentati F, Tounsi L, Djomdi D, et al. (2020) Bioactive polysaccharides from seaweeds. Molecules 25(14):3152

Holst JJ (2007) The physiology of glucagon-like peptide 1. Physiological reviews

Hong T, Zhao J, Dong M, Meng Y, Mu J, Yang Z (2012) Composition and bioactivity of polysaccharides from Inula britannica flower. International Journal of Biological Macromolecules 51(4):550-554

Hotamisligil G (2000) Molecular mechanisms of insulin resistance and the role of the adipocyte. International Journal of Obesity 24(4):S23-S27

Houeiss P, Luce S, Boitard C (2022) Environmental triggering of type 1 diabetes autoimmunity. Frontiers in Endocrinology 13:933965

Hu J, Pang W, Chen J, Bai S, Zheng Z, Wu X (2013a) Hypoglycemic effect of polysaccharides with different molecular weight of Pseudostellaria heterophylla. BMC Complementary and Alternative Medicine 13:1-9

Hu S, Chang Y, Wang J, Xue C, Li Z, Wang Y (2013b) Fucosylated chondroitin sulfate from sea cucumber in combination with rosiglitazone improved glucose metabolism in the liver of the insulin-resistant mice. Bioscience, Biotechnology, and Biochemistry 77(11):2263-2268

Hu S, Chang Y, Wang J, et al. (2013c) Fucosylated chondroitin sulfate from Acaudina molpadioides improves hyperglycemia via activation of PKB/GLUT4 signaling in skeletal muscle of insulin resistant mice. Food & Function 4(11):1639-1646

Hu S, Wang J, Xu H, Wang Y, Li Z, Xue C (2014a) Fucosylated chondroitin sulphate from sea cucumber inhibits high-fat-sucrose diet-induced apoptosis in mouse pancreatic islets via down-regulating mitochondrial signaling pathway. Journal of Functional Foods 7:517-526

Hu S, Xu H, Chen R, Wang J, Li Z, Xu J (2014b) Activation of PKB and ERK, but not PI3K, is involved in fucosylated chondroitin sulphate from Acaudina molpadioides induced glucose uptake. Journal of Functional Foods 10:385-396

Huang M, Wang F, Zhou X, Yang H, Wang Y (2015) Hypoglycemic and hypolipidemic properties of polysaccharides from Enterobacter cloacae Z0206 in KKAy mice. Carbohydrate polymers 117:91-98

Huang S, Pang D, Li X, et al. (2019) A sulfated polysaccharide from Gracilaria Lemaneiformis regulates cholesterol and bile acid metabolism in high-fat diet mice. Food & Function 10(6):3224-3236

Huang Z, Hu M, Peng X, Wang R, Song X, Yin J (2023) The protective effect of small black soybean (Vigna Mungo L.) polysaccharide on acetic acid-induced gastric ulcer in SD rats and its impact on gut microbiota and metabolites. Food Bioscience 56:103187

IDF (2021) IDF diabetes atlas. In. https://diabetesatlas.org/idfawp/resourcefiles/2021/07/IDF_Atlas_10th_Edition_2021.pdf Accessed July 14, 2023 2023

IDF (2025) IDF Diabetes Atlas. In: International Diabetes Federation. https://diabetesatlas.org/resources/idf-diabetes-atlas-2025/ Accessed April 17 2025

Ighodaro O, Akinloye O (2018) First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria journal of medicine 54(4):287-293

Im S-S, Kang S-Y, Kim S-Y, et al. (2005) Glucose-stimulated upregulation of GLUT2 gene is mediated by sterol response element–binding protein-1c in the hepatocytes. Diabetes 54(6):1684-1691

Inoguchi T, Li P, Umeda F, et al. (2000) High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C--dependent activation of NAD (P) H oxidase in cultured vascular cells. Diabetes 49(11):1939-1945

Islam S, Rahman S, Haque T, Sumon AH, Ahmed AM, Ali N (2020) Prevalence of elevated liver enzymes and its association with type 2 diabetes: A cross-sectional study in Bangladeshi adults. Endocrinology, diabetes & metabolism 3(2):e00116

Iynedjian P, Marie S, Gjinovci A, et al. (1995) Glucokinase and cytosolic phosphoenolpyruvate carboxykinase (GTP) in the human liver. Regulation of gene expression in cultured hepatocytes. The Journal of clinical investigation 95(5):1966-1973

Jadhav EB, Sankhla MS, Bhat RA, Bhagat D (2021) Microplastics from food packaging: An overview of human consumption, health threats, and alternative solutions. Environmental Nanotechnology, Monitoring & Management 16:100608

Jahromi MM, Eisenbarth GS (2007) Cellular and molecular pathogenesis of type 1A diabetes. Cellular and Molecular Life Sciences 64(7-8):865-872

Janero DR (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biology and Medicine 9(6):515540

Jelodar G, Mohammadi M, Akbari A, Nazifi S (2020) Cyclohexane extract of walnut leaves improves indices of oxidative stress, total homocysteine and lipids profiles in streptozotocin-induced diabetic rats. Physiological reports 8(1):e14348

Jena AB, Samal RR, Bhol NK, Duttaroy AK (2023) Cellular Red-Ox system in health and disease: The latest update. Biomedicine & Pharmacotherapy 162:114606

Jia R-B, Wu J, Li Z-R, et al. (2020) Structural characterization of polysaccharides from three seaweed species and their hypoglycemic and hypolipidemic activities in type 2 diabetic rats. International journal of biological macromolecules 155:1040-1049

Jiang H, Yang SQ, Chakka VP, et al. (2021) Purification and biological activities of enzymatically degraded Sargassum fusiforme polysaccharides. Chemistry & Biodiversity 18(3):e2000930

Jiang S, Young JL, Wang K, Qian Y, Cai L (2020) Diabetic-induced alterations in hepatic glucose and lipid metabolism: the role of type 1 and type 2 diabetes mellitus. Molecular medicine reports 22(2):603-611

Jin H, Zhang Y-J, Jiang J-X, et al. (2013) Studies on the extraction of pumpkin components and their biological effects on blood glucose of diabetic mice. Journal of food and drug analysis 21(2):184-189

Judi L, Toukan A, Khader Y, Ajlouni K, Khatib MA (2010) Prevalence of elevated hepatic transaminases among Jordanian patients with type 2 diabetes mellitus. Annals of Saudi medicine 30(1):25-32

Kajimoto Y, Kaneto H (2004) Role of Oxidative Stress in Pancreatic β-Cell Dysfunction. Annals of the New York Academy of Sciences 1011(1):168-176

Kanagasabapathy G, Kuppusamy UR, Abd Malek SN, Abdulla MA, Chua K-H, Sabaratnam V (2012) Glucan-rich polysaccharides from Pleurotus sajor-caju (Fr.) Singer prevents glucose intolerance, insulin resistance and inflammation in C57BL/6J mice fed a high-fat diet. BMC Complementary and Alternative Medicine 12:1-9

Kaul N, Ali S (2016) Genes, genetics, and environment in type 2 diabetes: implication in personalized medicine. DNA and Cell Biology 35(1):1-12

Kawai K, Hagiwara T (2018) Control of physical changes in food products. Survival strategies in extreme cold and desiccation: Adaptation mechanisms and their applications:385-399

Ke C, Li L (2023) Influence mechanism of polysaccharides induced Maillard reaction on plant proteins structure and functional properties: A review. Carbohydrate Polymers 302:120430

Ke Y, Yang J, Lin H Advances in the structural characterization and pharmacological activity of Salvia miltiorrhiza polysaccharides. Frontiers in Chemistry 13:1492533

Keran DA, Kumar PS, Venugopal V, Kumar R, Tarakanth K, Chinnappa A (2025) Degradation of polysaccharides during food processing Chemistry of Thermal and Non-Thermal Food Processing Technologies. Elsevier, p 461-501

Kerner W, Brückel J (2014) Definition, classification and diagnosis of diabetes mellitus. Experimental and clinical endocrinology & diabetes 122(07):384-386

Khandwal D, Patel S, Pandey AK, Mishra A (2025) A Comprehensive, Analytical Narrative Review of Polysaccharides from the Red Seaweed Gracilaria: Pharmaceutical Applications and Mechanistic Insights for Human Health. Nutrients 17(5):744

Kim MS, Polychronakos C (2005) Immunogenetics of type 1 diabetes. Hormone Research in Paediatrics 64(4):180-188

Knip M, Virtanen SM, Åkerblom HK (2010) Infant feeding and the risk of type 1 diabetes. The American journal of clinical nutrition 91(5):1506S-1513S

Koh HSA, Lu J, Zhou W (2020) Structural Dependence of Sulfated Polysaccharide for Diabetes Management: Fucoidan From Undaria pinnatifida Inhibiting α-Glucosidase More Strongly Than α-Amylase and Amyloglucosidase. Front Pharmacol 11:831 doi:10.3389/fphar.2020.00831

Koliaki C, Roden M (2013) Hepatic energy metabolism in human diabetes mellitus, obesity and non-alcoholic fatty liver disease. Molecular and Cellular Endocrinology 379(1-2):35-42

Kumar Bhateja P, Singh R (2014) Antidiabetic Activity of Acacia Tortilis (Forsk.) Hayne Ssp. Raddiana Polysaccharide on Streptozotocin-Nicotinamide Induced Diabetic Rats. BioMed research international 2014(1):572013

Lee H-G, Je J-G, Hwang J, et al. (2021) Comparision of antioxidant and anti-inflammatory activities of enzyme assisted hydrolysate from Ecklonia maxima blades and stipe. Fisheries and Aquatic Sciences 24(5):197-206

Leszek J, Trypka E, V Tarasov V, Md Ashraf G, Aliev G (2017) Type 3 diabetes mellitus: a novel implication of Alzheimers disease. Current Topics in Medicinal Chemistry 17(12):13311335

Li F, Zhang Y, Zhong Z (2011) Antihyperglycemic effect of Ganoderma lucidum polysaccharides on streptozotocin-induced diabetic mice. International journal of molecular sciences 12(9):6135-6145

Li HN, Zhao LL, Chen DQ (2020) Ganoderma lucidum polysaccharides ameliorates hepatic steatosis and oxidative stress in db/db mice via targeting nuclear factor E2 (erythroidderived 2)-related factor-2/heme oxygenase-1 (HO-1) pathway. Medical Science Monitor: international medical journal of experimental and clinical research 26:e921905-1

Li J, Li S, Liu S, et al. (2019) Pectic oligosaccharides hydrolyzed from citrus canning processing water by Fenton reaction and their antiproliferation potentials. International journal of biological macromolecules 124:1025-1032

Li S, Chen H, Wang J, Wang X, Hu B, Lv F (2015) Involvement of the PI3K/Akt signal pathway in the hypoglycemic effects of tea polysaccharides on diabetic mice. International Journal of Biological Macromolecules 81:967-974

Li Y, Liu Y, Liu S, et al. (2023) Diabetic vascular diseases: molecular mechanisms and therapeutic strategies. Signal transduction and targeted therapy 8(1):152

Li Y, Zheng Y, Zhang Y, et al. (2021) Brown algae carbohydrates: Structures, pharmaceutical properties, and research challenges. Marine drugs 19(11):620

Liao X, Yang L, Chen M, Yu J, Zhang S, Ju Y (2015) The hypoglycemic effect of a polysaccharide (GLP) from Gracilaria lemaneiformis and its degradation products in diabetic mice. Food & function 6(8):2542-2549

Lie BA, Todd JA, Pociot F, et al. (1999) The predisposition to type 1 diabetes linked to the human leukocyte antigen complex includes at least one non–class II gene. The American Journal of Human Genetics 64(3):793-800

Lin W, Wang W, Liao D, et al. (2015) Polysaccharides from Enteromorpha prolifera improve glucose metabolism in diabetic rats. Journal of Diabetes Research 2015(1):675201

Liu C-M, Ma J-Q, Sun Y-Z (2012) Puerarin protects rat kidney from lead-induced apoptosis by modulating the PI3K/Akt/eNOS pathway. Toxicology and Applied Pharmacology 258(3):330-342

Liu Y, Sun J, Rao S, et al. (2013a) Antidiabetic activity of mycelia selenium-polysaccharide from Catathelasma ventricosum in STZ-induced diabetic mice. Food and Chemical Toxicology 62:285-291

Liu Y, Sun J, Rao S, Su Y, Yang Y (2013b) Antihyperglycemic, antihyperlipidemic and antioxidant activities of polysaccharides from Catathelasma ventricosum in streptozotocin-induced diabetic mice. Food and Chemical Toxicology 57:39-45

Liu Y, Wan L, Xiao Z, Wang J, Wang Y, Chen J (2013c) Antidiabetic activity of polysaccharides from tuberous root of Liriope spicata var. prolifera in KKAy mice. Evidence-Based Complementary and Alternative Medicine 2013(1):349790

Liu Y, Yang L, Zhang Y, et al. (2020) Dendrobium officinale polysaccharide ameliorates diabetic hepatic glucose metabolism via glucagon-mediated signaling pathways and modifying liver-glycogen structure. Journal of Ethnopharmacology 248:112308

Lovegrove A, Edwards C, De Noni I, et al. (2017) Role of polysaccharides in food, digestion, and health. Critical Reviews in Food Science and Nutrition 57(2):237-253

Lu S-Y, Tan K, Zhong S, Cheong K-L (2023) Marine algal polysaccharides as future potential constituents against non-alcoholic steatohepatitis. International Journal of Biological Macromolecules 250:126247

Luo X, Wu J, Jing S, Yan L-J (2016) Hyperglycemic stress and carbon stress in diabetic glucotoxicity. Aging and Disease 7(1):90-110

Ma X, Zhou F, Chen Y, et al. (2014) A polysaccharide from Grifola frondosa relieves insulin resistance of HepG2 cell by Akt-GSK-3 pathway. Glycoconjugate journal 31:355-363

Ma Y, Lee G, Heo S-Y, Roh Y-S (2021) Oxidative stress is a key modulator in the development of nonalcoholic fatty liver disease. Antioxidants 11(1):91

Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ (2010) Epidemiology of type 1 diabetes. Endocrinology and Metabolism Clinics 39(3):481-497

Madiraju AK, Erion DM, Rahimi Y, et al. (2014) Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. Nature 510(7506):542-546

Mafe AN, Edo GI, Makia RS, et al. (2024) A review on food spoilage mechanisms, food borne diseases and commercial aspects of food preservation and processing. Food Chemistry Advances 5:100852

Mahato AK, Bhattacharya S, Shanthi N (2011) Design, Synthesis and Glucose-6-Phosphatase Inhibitory Activity of Diaminoguanidine Analogues of 3-Guanidinopropionic Acid and Amino Substituted (Pyridin-2-Yl) thiourea Derivatives. Journal of Pharmaceutical Science Research 3:896-902

Mahmood AA, Zainal AA, Khalaf MM (2021) Cholinesterase activity in non-alcoholic-fatty liver disease in diabetic patients taking oral antidiabetic drugs. Pharmacology 25

Mallet V, Parlati L, Martinino A, et al. (2022) Burden of liver disease progression in hospitalized patients with type 2 diabetes mellitus. Journal of Hepatology 76(2):265-274

Martin M, Marais R (2012) Metformin: a diabetes drug for cancer, or a cancer drug for diabetics? Journal of Clinical Oncology 30(21):2698-2700

Mayerson AB, Hundal RS, Dufour S, et al. (2002) The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. Diabetes 51(3):797-802

Mba CM, Mbanya JC (2020) Challenges and economic burden of diabetes in Africa Obesity and Diabetes: Scientific Advances and Best Practice. Springer, p 21-34

Mendez-Sanchez N, Cruz-Ramon VC, Ramirez-Perez OL, Hwang JP, Barranco-Fragoso B, CordovaGallardo J (2018) New aspects of lipotoxicity in nonalcoholic steatohepatitis. International journal of molecular sciences 19(7):2034

Meyerstein D (2021) Re-examining Fenton and Fenton-like reactions. Nature Reviews Chemistry 5(9):595-597

Miller RA, Chu Q, Xie J, Foretz M, Viollet B, Birnbaum MJ (2013) Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature 494(7436):256-260

Mobasheri L, Ahadi M, Namdar AB, et al. (2023) Pathophysiology of diabetic hepatopathy and molecular mechanisms underlying the hepatoprotective effects of phytochemicals. Biomedicine & Pharmacotherapy 167:115502

Mohamed AM, El-Sharkawy F, Ahmed S, Aziz W, Badary OA (2009) Glycemic control and therapeutic effect of Nigella sativa and Curcuma longa on rats with streptozotocininduced diabetic hepatopathy.

Mohammadi M, Aelaei M, Saidi M (2021) Pre-harvest spray of GABA and spermine delays postharvest senescence and alleviates chilling injury of gerbera cut flowers during cold storage. Scientific Reports 11(1):14166

Mohammadigheisar M, Shouldice V, Sands J, Lepp D, Diarra M, Kiarie E (2020) Growth performance, breast yield, gastrointestinal ecology and plasma biochemical profile in broiler chickens fed multiple doses of a blend of red, brown and green seaweeds. British Poultry Science 61(5):590-598

Mooradian AD (1988) Diabetic complications of the central nervous system. Endocrine Reviews 9(3):346-356

Mphasha MH, Stubbendorff A (2025) Challenging Diabetes in Rural Areas: Aspects of Nutrition Handbook of Public Health Nutrition: International, National, and Regional Perspectives. Springer, p 1-23

Msomi NZ, Erukainure OL, Salau VF, Olofinsan KA, Islam MS (2023) 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. Food Science and Human Wellness 12(6):2052-2060

Murakami S, Hirazawa C, Ohya T, et al. (2021) The edible brown seaweed Sargassum horneri (Turner) C. Agardh ameliorates high-fat diet-induced obesity, diabetes, and hepatic steatosis in mice. Nutrients 13(2):551

Murea M, Ma L, Freedman BI (2012) Genetic and environmental factors associated with type 2 diabetes and diabetic vascular complications. Review of Diabetic Studies 9(1):6

Nagahawatta D, Liyanage N, Jayawardhana H, Lee H-G, Jayawardena TU, Jeon Y-J (2022) Anti-fine dust effect of fucoidan extracted from Ecklonia maxima leaves in macrophages via inhibiting inflammatory signaling pathways. Marine Drugs 20(7):413

Nguyen MT, Lian A, Guilford FT, Venketaraman V (2025) A Literature Review of Glutathione Therapy in Ameliorating Hepatic Dysfunction in Non-Alcoholic Fatty Liver Disease. Biomedicines 13(3):644

Noble JA, Valdes AM (2011) Genetics of the HLA region in the prediction of type 1 diabetes. Current diabetes reports 11:533-542

Oboh G, Ademosun AO (2011) Shaddock peels (Citrus maxima) phenolic extracts inhibit αamylase, α-glucosidase and angiotensin I-converting enzyme activities: A nutraceutical approach to diabetes management. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 5(3):148-152

Oguntibeju OO (2019) Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links. International journal of physiology, pathophysiology and pharmacology 11(3):45

Olasehinde TA, Mabinya LV, Olaniran AO, Okoh AI (2019a) Chemical characterization, antioxidant properties, cholinesterase inhibitory and anti-amyloidogenic activities of sulfated polysaccharides from some seaweeds. Bioactive Carbohydrates and Dietary Fibre 18:100182

Olasehinde TA, Olaniran AO, Okoh AI (2019b) Macroalgae as a valuable source of naturally occurring bioactive compounds for the treatment of Alzheimer's disease. Marine drugs 17(11):609

Olasehinde TA, Olaniran AO, Okoh AI (2020) Sulfated polysaccharides of some seaweeds exhibit neuroprotection via mitigation of oxidative stress, cholinergic dysfunction and inhibition of Zn–induced neuronal damage in HT-22 cells. BMC complementary medicine and therapies 20:1-10

Opara UL, Pathare PB (2024) Mechanical damage of fresh produce: An overview. Mechanical Damage in Fresh Horticultural Produce: Measurement, Analysis and Control:1-19

Osadebe PO, Odoh EU, Uzor PF (2015) Oral Anti-Diabetic Agents-Review and Updates. British Journal of Medicine & Medical Research 5(2):134-159

Otero P, Carpena M, Garcia-Oliveira P, et al. (2023) Seaweed polysaccharides: Emerging extraction technologies, chemical modifications and bioactive properties. Critical Reviews in Food Science and Nutrition 63(13):1901-1929

Owen MR, Doran E, Halestrap AP (2000) Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochemical journal 348(3):607-614

Oyinloye TM, Yoon WB (2020) Effect of freeze-drying on quality and grinding process of food produce: A review. Processes 8(3):354

Ozougwu J, Obimba K, Belonwu C, Unakalamba C (2013) The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physiol Pathophysiol 4(4):46-57

Pan D, Wang L, Chen C, Hu B, Zhou P (2015) Isolation and characterization of a hyperbranched proteoglycan from Ganoderma lucidum for anti-diabetes. Carbohydrate Polymers 117:106-114

Pang Z, Lu Y, Zhou G, et al. (2024) MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. Nucleic acids research 52(W1):W398-W406

Pathare PB, Al-Dairi M (2022) Effect of mechanical damage on the quality characteristics of banana fruits during short-term storage. Discover Food 2(1):4

Pengzhan Y, Ning L, Xiguang L, Gefei Z, Quanbin Z, Pengcheng L (2003) Antihyperlipidemic effects of different molecular weight sulfated polysaccharides from Ulva pertusa (Chlorophyta). Pharmacological Research 48(6):543-549

Pereira R, Yarish C (2008) Mass production of marine macroalgae In: Jørgensen, SE, Fath, BD (Eds.), Encyclopedia of Ecology. Academic Press, Oxford

Perkins RM, Yuan CM, Welch PG (2006) Dipsogenic diabetes insipidus: report of a novel treatment strategy and literature review. Clinical and Experimental Nephrology 10(1):6367

Peroni-Okita FH, Cardoso MB, Agopian RG, et al. (2013) The cold storage of green bananas affects the starch degradation during ripening at higher temperature. Carbohydrate polymers 96(1):137-147

Petersen MC, Shulman GI (2018) Mechanisms of insulin action and insulin resistance. Physiological Reviews

Pi J, Zhang Q, Fu J, et al. (2010) ROS signaling, oxidative stress and Nrf2 in pancreatic beta-cell function. Toxicology and Applied Pharmacology 244(1):77-83

Pillay LR, Olasehinde TA, Olofinsan K, et al. (2025) The impact of sulphated polysaccharides from Ecklonia maxima on carbohydrate hydrolyzing enzymes, muscle glucose uptake and intestinal glucose absorption ex vivo. Algal Research 85:103831

Pillay LR, Olasehinde TA, Olofinsan KA, Erukainure OL, Islam MS, Olaniran AO (2024) Antidiabetic potentials of crude and purified sulphated polysaccharides isolated from Gracilaria gracilis, a seaweed from South Africa. Heliyon 10(15)

Pirot P, Cardozo AK, Eizirik DL (2008) Mediators and mechanisms of pancreatic beta-cell death in type 1 diabetes. Arquivos Brasileiros de Endocrinologia & Metabologia 52(2):156-165

Poitout V, Robertson RP (2008) Glucolipotoxicity: fuel excess and β-cell dysfunction. Endocrine reviews 29(3):351-366

Pomin VH (2012) Seaweed: Ecology, nutrient composition and medicinal uses. (No Title)

Ponce NM, Stortz CA (2020) A comprehensive and comparative analysis of the fucoidan compositional data across the Phaeophyceae. Frontiers in Plant Science 11:556312

Pratley RE, Salsali A (2007) Inhibition of DPP-4: a new therapeutic approach for the treatment of type 2 diabetes. Current Medical Research and Opinion 23(4):919-931

Qaid MM, Abdelrahman MM (2016) Role of insulin and other related hormones in energy metabolism—A review. Cogent Food & Agriculture 2(1):1267691 doi:10.1080/23311932.2016.1267691

Radzki W, Ziaja-Sołtys M, Nowak J, et al. (2016) Effect of processing on the content and biological activity of polysaccharides from Pleurotus ostreatus mushroom. LWT-Food Science and Technology 66:27-33

Ren C, Zhang Y, Cui W, et al. (2015) A polysaccharide extract of mulberry leaf ameliorates hepatic glucose metabolism and insulin signaling in rats with type 2 diabetes induced by high fatdiet and streptozotocin. International journal of biological macromolecules 72:951-959

Ren D, Zhao Y, Nie Y, Yang N, Yang X (2014) Hypoglycemic and hepatoprotective effects of polysaccharides from Artemisia sphaerocephala Krasch seeds. International journal of biological macromolecules 69:296-306

Ren S, Wang J, Wang Y, et al. (2025) Oxidative stress and type 2 diabetes: a review of lactic acid bacteria as potential prophylactic and therapeutic interventions. Food Science and Biotechnology:1-14

Rines AK, Sharabi K, Tavares CD, Puigserver P (2016) Targeting hepatic glucose metabolism in the treatment of type 2 diabetes. Nature reviews Drug discovery 15(11):786-804

Rolo AP, Palmeira CM (2006) Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. Toxicology and Applied Pharmacology 212(2):167-178

Saborio P, Tipton GA, Chan J (2000) Diabetes insipidus. Pediatrics in Review 21(4):122-129

Schmid AI, Szendroedi J, Chmelik M, Krššák M, Moser E, Roden M (2011) Liver ATP synthesis is lower and relates to insulin sensitivity in patients with type 2 diabetes. Diabetes care 34(2):448-453

Schmidt SJ, Lee JW (2009) How does the freezer burn our food? Journal of Food Science Education 8(2):45-52

Schutte M, Hayward S, Manley M (2024) Nonenzymatic browning and antioxidant properties of thermally treated cereal grains and end products. Journal of Food Biochemistry 2024(1):3865849

Shabalala SC, Johnson R, Basson AK, et al. (2022) Detrimental effects of lipid peroxidation in type 2 diabetes: Exploring the neutralizing influence of antioxidants. Antioxidants 11(10):2071

Sharabi K, Tavares CD, Rines AK, Puigserver P (2015) Molecular pathophysiology of hepatic glucose production. Molecular aspects of medicine 46:21-33

Shaw K (2017) 'Type 3'diabetes: a brain insulin-resistant state linked to Alzheimer's disease. Practical Diabetes 34(6):187-188

Silberfeld T, Leigh JW, Verbruggen H, Cruaud C, De Reviers B, Rousseau F (2010) A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the "brown algal crown radiation". Molecular phylogenetics and evolution 56(2):659-674

Silva M, Vida M, Ramos AC, Lidon FJ, Reboredo FH, Gonçalves EM (2025) Storage Temperature Effect on Quality and Shelf-Life of Hericium erinaceus Mushroom. Horticulturae 11(2):158

Singh RP (2024) Kidney bean starch: composition, structure, properties, and modifications Non-Conventional Starch Sources. Elsevier, p 439-465

Snezhkina AV, Kudryavtseva AV, Kardymon OL, et al. (2019) ROS generation and antioxidant defense systems in normal and malignant cells. Oxidative medicine and cellular longevity 2019(1):6175804

Song P, Huang Y, Li J, et al. (2024) The influence of processing technologies on the biological activity of carbohydrates in food. Food Chemistry: X 23:101590

Soyer A, Özalp B, Dalmış Ü, Bilgin V (2010) Effects of freezing temperature and duration of frozen storage on lipid and protein oxidation in chicken meat. Food chemistry 120(4):1025-1030

Stankov K, Benc D, Draskovic D (2013) Genetic and epigenetic factors in etiology of diabetes mellitus type 1. Pediatrics 132(6):1112-1122

Su L-J, Zhang J-H, Gomez H, et al. (2019) Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. Oxidative medicine and cellular longevity 2019(1):5080843

Sun C, Chen Y, Li X, Tai G, Fan Y, Zhou Y (2014) Anti-hyperglycemic and anti-oxidative activities of ginseng polysaccharides in STZ-induced diabetic mice. Food & Function 5(5):845-848

Sun L, Zhu Z, Sun D-W (2023) Regulating ice formation for enhancing frozen food quality: Materials, mechanisms and challenges. Trends in Food Science & Technology 139:104116

Sun X-Y, Zhang H, Liu J, Ouyang J-M (2019) Repair activity and crystal adhesion inhibition of polysaccharides with different molecular weights from red algae Porphyra yezoensis against oxalate-induced oxidative damage in renal epithelial cells. Food & Function 10(7):3851-3867

Suzanne M (2014) Type 3 diabetes is sporadic Alzheimer' s disease: Mini-review. European Neuropsychopharmacology 24(12):1954-1960

Suzuki A, Lindor K, St Saver J, et al. (2005) Effect of changes on body weight and lifestyle in nonalcoholic fatty liver disease. Journal of hepatology 43(6):1060-1066

Szablewski L (2014) Role of immune system in type 1 diabetes mellitus pathogenesis. International immunopharmacology 22(1):182-191

Szeligowska N, Cholewińska P, Smoliński J, et al. (2022) Glutathione S-transferase (GST) and cortisol levels vs. microbiology of the digestive system of sheep during lambing. BMC Veterinary Research 18(1):107

Thrasher J (2017) Pharmacologic management of type 2 diabetes mellitus: available therapies. American Journal of Cardiology 120(1):S4-S16

Tolman KG, Fonseca V, Dalpiaz A, Tan MH (2007) Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. Diabetes care 30(3):734-743

Tripathi BK, Srivastava AK (2006) Diabetes mellitus: Complications and therapeutics. Medical Science Monitor 12(7):RA130-RA147

Ullah J, Takhar PS, Sablani SS (2014) Effect of temperature fluctuations on ice-crystal growth in frozen potatoes during storage. LWT-Food Science and Technology 59(2):1186-1190

Utzschneider KM, Kahn SE (2006) The role of insulin resistance in nonalcoholic fatty liver disease. The Journal of Clinical Endocrinology & Metabolism 91(12):4753-4761

Vallon V, Nakagawa T (2021) Renal tubular handling of glucose and fructose in health and disease. Comprehensive Physiology 12(1):2995

Vallon V, Thomson SC (2017) Targeting renal glucose reabsorption to treat hyperglycaemia: the pleiotropic effects of SGLT2 inhibition. Diabetologia 60(2):215-225

Van der Sman R (2020) Impact of processing factors on quality of frozen vegetables and fruits. Food Engineering Reviews 12:399-420

Vilas-Boas EA, Almeida DC, Roma LP, Ortis F, Carpinelli AR (2021) Lipotoxicity and β-cell failure in type 2 diabetes: Oxidative stress linked to NADPH oxidase and ER stress. Cells 10(12):3328

Wade R, Augyte S, Harden M, Nuzhdin S, Yarish C, Alberto F (2020) Macroalgal germplasm banking for conservation, food security, and industry. PLoS biology 18(2):e3000641

Wang C, Gao X, Santhanam RK, et al. (2018) Effects of polysaccharides from Inonotus obliquus and its chromium (III) complex on advanced glycation end-products formation, αamylase, α-glucosidase activity and H2O2-induced oxidative damage in hepatic L02 cells. Food and Chemical Toxicology 116:335-345

Wang D, Xiao H, Lyu X, Chen H, Wei F (2023) Lipid oxidation in food science and nutritional health: A comprehensive review. Oil Crop Science 8(1):35-44

Wang F, Yang H, Wang Y (2013a) Structure characterization of a fucose-containing exopolysaccharide produced by Enterobacter cloacae Z0206. Carbohydrate polymers 92(1):503-509

Wang J, Jin W, Zhang W, Hou Y, Zhang H, Zhang Q (2013b) Hypoglycemic property of acidic polysaccharide extracted from Saccharina japonica and its potential mechanism. Carbohydrate polymers 95(1):143-147

Wang L-Y, Wang Y, Xu D-S, Ruan K-F, Feng Y, Wang S (2012) MDG-1, a polysaccharide from Ophiopogon japonicus exerts hypoglycemic effects through the PI3K/Akt pathway in a diabetic KKAy mouse model. Journal of Ethnopharmacology 143(1):347-354

Wang L, Wu H, Chang N, Zhang K (2011) Anti-hyperglycemic effect of the polysaccharide fraction isolated from mactra veneriformis. Frontiers of Chemical Science and Engineering 5:238244

Wang Y (2017) Study on standardization method of Astragalus polysaccharide reference substance. Zhongcaoyao:4897-4903

Wei Z, Weng S, Wang L, Mao Z (2018) Mechanism of Astragalus polysaccharides in attenuating insulin resistance in Rats with type 2 diabetes mellitus via the regulation of liver microRNA-203a-3p. Molecular Medicine Reports 17(1):1617-1624

Wen L, Zhang Y, Sun-Waterhouse D, You L, Fu X (2017) Advantages of the polysaccharides from Gracilaria lemaneiformis over metformin in antidiabetic effects on streptozotocininduced diabetic mice. RSC advances 7(15):9141-9151

Westcott F, Dearlove DJ, Hodson L (2024) Hepatic fatty acid and glucose handling in metabolic disease: potential impact on cardiovascular disease risk. Atherosclerosis 394:117237

Wiklund P, Zhang X, Pekkala S, Autio R, Kong L, Yang Y (2018) Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. Sci Rep. 2016; 6: 1–11. American Journal of Epidemiology 187(8):1662-1669

Wilcox G (2005) Insulin and insulin resistance. Clinical Biochemist Reviews 26(2):19-39

Williams R, Karuranga S, Malanda B, et al. (2020) Global and regional estimates and projections of diabetes-related health expenditure: Results from the International Diabetes Federation Diabetes Atlas. Diabetes Research and Clinical Practice 162:108072

Woo KS, Kim HY, Hwang IG, Lee SH, Jeong HS (2015) Characteristics of the thermal degradation of glucose and maltose solutions. Preventive nutrition and food science 20(2):102

Wu J, Shi S, Wang H, Wang S (2016) Mechanisms underlying the effect of polysaccharides in the treatment of type 2 diabetes: A review. Carbohydrate polymers 144:474-494

Xiang Y, Zhang M, Jiang D, Su Q, Shi J (2023) The role of inflammation in autoimmune disease: a therapeutic target. Frontiers in Immunology 14:1267091

Xiao C, Wu Q-P, Cai W, Tan J-B, Yang X-B, Zhang J-M (2012) Hypoglycemic effects of Ganoderma lucidum polysaccharides in type 2 diabetic mice. Archives of pharmacal research 35:17931801

Xiao C, Wu Q, Xie Y, Zhang J, Tan J (2015) Hypoglycemic effects of Grifola frondosa (Maitake) polysaccharides F2 and F3 through improvement of insulin resistance in diabetic rats. Food & function 6(11):3567-3575

Xiao X, Erukainure OL, Islam MS (2023) Red honeybush (Cyclopia genistoides) tea mitigates oxidative imbalance and hyperlipidemia, while improving glucose homeostasis in type 2 diabetic rats. Food Science and Human Wellness 12(6):2029-2039

Xiao Zq, Wang Yl, Gan Sr, Chen Jc (2014) Polysaccharides from Liriopes Radix ameliorates hyperglycemia via various potential mechanisms in diabetic rats. Journal of the Science of Food and Agriculture 94(5):975-982

Xu Bw, Li Ss, Ding Wl, et al. (2025) From structure to function: A comprehensive overview of polysaccharide roles and applications. Food Frontiers 6(1):15-39

Xu L, Yang F, Wang J, Huang H, Huang Y (2015a) Anti-diabetic effect mediated by Ramulus mori polysaccharides. Carbohydrate Polymers 117:63-69

Xu W, Zhou Q, Yin J-j, Yao Y, Zhang J-l (2015b) Anti-diabetic effects of polysaccharides from Talinum triangulare in streptozotocin (STZ)-induced type 2 diabetic male mice. International Journal of Biological Macromolecules 72:575-579

Xu Y, Niu X, Liu N, et al. (2018) Characterization, antioxidant and hypoglycemic activities of degraded polysaccharides from blackcurrant (Ribes nigrum L.) fruits. Food Chemistry 243:26-35

Xu Y, Zhang X, Yan X-H, et al. (2019) Characterization, hypolipidemic and antioxidant activities of degraded polysaccharides from Ganoderma lucidum. International journal of biological macromolecules 135:706-716

Yang X, Li A, Li X, Sun L, Guo Y (2020) An overview of classifications, properties of food polysaccharides and their links to applications in improving food textures. Trends in Food Science & Technology 102:1-15

Yazdi HB, Hojati V, Shiravi A, Hosseinian S, Vaezi G, Hadjzadeh M-A-R (2019) Liver dysfunction and oxidative stress in streptozotocin-induced diabetic rats: protective role of Artemisia turanica. Journal of pharmacopuncture 22(2):109

Ye M, Qiu T, Peng W, Chen W-x, Ye Y-w, Lin Y-r (2011) Purification, characterization and hypoglycemic activity of extracellular polysaccharides from Lachnum calyculiforme. Carbohydrate Polymers 86(1):285-290

Yu A-N, Li Y, Yang Y, Yu K (2017) The browning kinetics of the non-enzymatic browning reaction in L-ascorbic acid/basic amino acid systems. Food Science and Technology 38:537-542

Yu J, Tseng Y, Pham K, Liu M, Beckles DM (2022) Starch and sugars as determinants of postharvest shelf life and quality: some new and surprising roles. Current opinion in biotechnology 78:102844

Yu JG, Javorschi S, Hevener AL, et al. (2002) The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. Diabetes 51(10):29682974

Yu Z, Yang Z, da Silva JAT, Luo J, Duan J (2019) Influence of low temperature on physiology and bioactivity of postharvest Dendrobium officinale stems. Postharvest Biology and Technology 148:97-106

Zhang C, Jia J, Zhang P, et al. (2022) Fucoidan from Laminaria japonica ameliorates type 2 diabetes mellitus in association with modulation of gut microbiota and metabolites in streptozocintreated mice. Foods 12(1):33

Zhang J-G, Liu Q, Liu Z-L, Li L, Yi L-t (2015a) Antihyperglycemic activity of Anoectochilus roxburghii polysaccharose in diabetic mice induced by high-fat diet and streptozotocin. Journal of Ethnopharmacology 164:180-185

Zhang J, Meng G, Zhang C, et al. (2015b) The antioxidative effects of acidic-, alkalic-, and enzymatic-extractable mycelium zinc polysaccharides by Pleurotus djamor on liver and kidney of streptozocin-induced diabetic mice. BMC complementary and alternative medicine 15:1-12

Zhang L, Liu Y, Ke Y, et al. (2018) Antidiabetic activity of polysaccharides from Suillellus luridus in streptozotocin-induced diabetic mice. International journal of biological macromolecules 119:134-140

Zhang W, Gao J, Lu L, et al. (2021) Intracellular GSH/GST antioxidants system change as an earlier biomarker for toxicity evaluation of iron oxide nanoparticles. NanoImpact 23:100338

Zhang W, Zheng L, Zhang Z, Hai C-X (2012) Protective effect of a water-soluble polysaccharide from Salvia miltiorrhiza Bunge on insulin resistance in rats. Carbohydrate Polymers 89(3):890-898

Zhang X, Wang J, Zhang T, et al. (2024) Updated Progress on Polysaccharides with Anti-Diabetic Effects through the Regulation of Gut Microbiota: Sources, Mechanisms, and Structure–Activity Relationships. Pharmaceuticals 17(4):456 doi:10.3390/ph17040456

Zhang X, Yang S, Chen J, Su Z (2019) Unraveling the regulation of hepatic gluconeogenesis. Frontiers in Endocrinology 9:802

Zhang Y, Ren C, Lu G, et al. (2014) Purification, characterization and anti-diabetic activity of a polysaccharide from mulberry leaf. Regulatory Toxicology and Pharmacology 70(3):687695

Zhao L, Lan Q, Huang Z, Ouyang L, Zeng F (2011) Antidiabetic effect of a newly identified component of Opuntia dillenii polysaccharides. Phytomedicine 18(8-9):661-668

Zhao R, Qiu B, Li Q, et al. (2014) LBP-4a improves insulin resistance via translocation and activation of GLUT4 in OLETF rats. Food & Function 5(4):811-820

Zhao T, Mao G-h, Zhang M, et al. (2013) Anti-diabetic effects of polysaccharides from ethanolinsoluble residue of Schisandra chinensis (Turcz.) Baill on alloxan-induced diabetic mice. chemical research in chinese universities 29:99-102

Zhao Z, Wang L, Ruan Y, et al. (2023) Physicochemical properties and biological activities of polysaccharides from the peel of Dioscorea opposita Thunb. extracted by four different methods. Food Science and Human Wellness 12(1):130-139

Zheng J, Yang B, Yu Y, Chen Q, Huang T, Li D (2012) Ganoderma lucidum polysaccharides exert anti-hyperglycemic effect on streptozotocin-induced diabetic rats through affecting βcells. Combinatorial chemistry & high throughput screening 15(7):542-550

Zheng Y, Liu T, Wang Z, Xu Y, Zhang Q, Luo D (2018) Low molecular weight fucoidan attenuates liver injury via SIRT1/AMPK/PGC1α axis in db/db mice. International Journal of Biological Macromolecules 112:929-936

Zhong Q-W, Zhou T-S, Qiu W-H, et al. (2021) Characterization and hypoglycemic effects of sulfated polysaccharides derived from brown seaweed Undaria pinnatifida. Food Chemistry 341:128148

Zhou J, Xu G, Yan J, et al. (2015a) Rehmannia glutinosa (Gaertn.) DC. polysaccharide ameliorates hyperglycemia, hyperlipemia and vascular inflammation in streptozotocin-induced diabetic mice. Journal of ethnopharmacology 164:229-238

Zhou J, Yan J, Bai Z, Li K, Huang K (2015b) Hypoglycemic activity and potential mechanism of a polysaccharide from the loach in streptozotocin-induced diabetic mice. Carbohydrate polymers 121:199-206

Zhou Q-L, Wang Z, Chen W-T, et al. (2024) The structural characteristics, biological activities and mechanisms of bioactive brown seaweed polysaccharides: A review. Journal of Functional Foods 119:106303

Zhu H, Bai M, Xie X, et al. (2022) Impaired amino acid metabolism and its correlation with diabetic kidney disease progression in type 2 diabetes mellitus. Nutrients 14(16):3345

Zhu J, Liu W, Yu J, et al. (2013a) Characterization and hypoglycemic effect of a polysaccharide extracted from the fruit of Lycium barbarum L. Carbohydrate polymers 98(1):8-16

Zhu K-X, Nie S-P, Li C, Gong D, Xie M-Y (2014) Ganoderma atrum polysaccharide improves aortic relaxation in diabetic rats via PI3K/Akt pathway. Carbohydrate polymers 103:520-527

Zhu K, Nie S, Gong D, Xie M (2016) Effect of polysaccharide from Ganoderma atrum on the serum metabolites of type 2 diabetic rats. Food Hydrocolloids 53:31-36

Zhu K, Nie S, Li C, et al. (2013b) A newly identified polysaccharide from Ganoderma atrum attenuates hyperglycemia and hyperlipidemia. International journal of biological macromolecules 57:142-150

Zhu Z, Zhou Q, Sun D-W (2019) Measuring and controlling ice crystallization in frozen foods: A review of recent developments. Trends in Food Science & Technology 90:13-25

Zorena K, Michalska M, Kurpas M, Jaskulak M, Murawska A, Rostami S (2022) Environmental factors and the risk of developing type 1 diabetes—old disease and new data. Biology 11(4):608

Zou M-Y, Nie S-P, Yin J-Y, Xie M-Y (2020) Ascorbic acid induced degradation of polysaccharide from natural products: a review. International Journal of Biological Macromolecules 151:483-491

CHAPTER 5

Influence of Storage and Temperature on the Stability and Antidiabetic Properties of Sulphated Polysaccharides from Red (*Gracilaria gracilis*) and Brown (*Ecklonia maxima*) Seaweeds

[1]Lethiwe B. Mpungose, [1]Tosin A. Olasasehinde, [1,2]Ochuko L. Erukainure, [2]Md. Shahidul Islam, [1]Ademola O. Olaniran*

1. Department of Microbiology, School of Life Sciences, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa.
2. Department of Biochemistry, School of Life Sciences, University of Kwazulu-Natal, Westville Campus, Durban 4000, South Africa.

***Correspondence:** olanirana@ukzn.ac.za

Abstract

Storage conditions play an influential role on the stability of polysaccharides, affecting their physicochemical properties and biological activities. In the present study, the effect of storage temperature and time on the stability and antidiabetic properties of sulphated polysaccharide (SPs) from *Gracilaria gracilis* and *Ecklonia maxima* were investigated. SPs from *G. gracilis* and *E. maxima* were stored at 4, 25 and 37 ℃ for 0 - 6 months. The SPs were analyzed for total glucose and sulphate levels, α-glucosidase, and α-amylase inhibitory activities. There were no significant changes in the glucose and sulphate levels of SPs stored at 4 and 25 °C. However, these levels were significantly reduced in SPs stored at 37 °C, at the 5th and 6th month. Storage at 4 and 25 °C, had no significant effect on the α-glucosidase, and α-amylase inhibitory activities of SPs throughout the storage period. However, there was a time dependent decline in the inhibitory activities at 37 °C. These results suggest temperature dependent instability and decreased antidiabetic activities of SPs from *G. gracilis* and *E. maxima* which is influenced by high temperature and longer storage time. This is depicted by decreased glucose and sulphate levels and suppressed activities of α-glucosidase and α-amylase activities when stored above room temperature for more than four months.

Keywords: Antidiabetes; Brown seaweeds; Shelf-life; stability testing; Sulphated polysaccharides; and Red seaweeds.

5.1 Introduction

Seaweeds, also classified as marine macroalgae, are the major source of sulphated polysaccharides with significant chemical and biological properties (Kumar Bhateja and Singh 2014). Among these seaweeds are the red **(***Gracilaria* species) and brown (*Ecklonia* species). Red seaweeds belong to the genus, *Gracilaria* and are majorly found in Chile and China, as well as in other countries like Taiwan, South Africa, Namibia, the Philippines, and Vietnam (Pereira and Yarish 2008). They are bushy in appearance, with a cylindrically compressed and irregularly branched thallus (Pereira and Yarish 2008). They are the primary sources of agar and are mainly consumed in the Caribbean, Southeast Asia, Japan, and Hawaii (Khandwal et al. 2025). *Gracilaria* species have been reported for their polysaccharide constituents which consist mainly of floridean starch, carrageenan, agar and galactans. The medicinal properties of *Gracilaria* spp. polysaccharides have been reported to include antiinflammation, antioxidant, immunomodulation, neuroprotection, antidiabetes and antiobesity (Hentati et al. 2020; Olasehinde et al. 2019b; Pillay et al. 2024). *Gracilaria gracilis* is a red seaweed specie rich in sulphated polysaccharides (SPs). Its monosaccharide composition consist of glucose, mannuronic acid, galactose, arabinose, xylose, and glucuronic acid (185 : 141 : 21 : 12 : 1 : 142) (Pillay et al. 2024). The antidiabetic properties of *G. gracilis* SPs have been demonstrated by their ability to promote muscle glucose uptake, and inhibition of carbohydrate digestive enzyme activities and intestinal glucose absorption (Pillay et al. 2024).

Brown seaweeds belong to the class, Phaeophyceae and are among the major contributors to coastal environments' biomass (Silberfeld et al. 2010). Brown seaweed polysaccharides are highly sulphated and mainly consist of laminarin, fucoidans, alginate and fucans (de Jesus Raposo et al. 2015; Hentati et al. 2020). Brown seaweed polysaccharides have been reported for their biological activities which include antiinflammation, antioxidant, anticancer, antiobesogenic,

hepatoprotection and immunomodulation (Borazjani et al. 2018; Jiang et al. 2021; Murakami et al. 2021; Zhou et al. 2024). *Ecklonia maxima* is a specie of brown seaweed endemic to South Africa and has been reported for its rich content of sulphated polysaccharides (Pillay et al. 2025). *Ecklonia maxima* polysaccharides have been reported for their potent inhibition of glucose metabolizing enzymes and intestinal glucose absorption, as well as promotion of muscle glucose uptake (Daub et al. 2020; Pillay et al. 2025) which demonstrates its antidiabetic properties. Fucoidan EMLF7 extracted from *E. maxima* has been reported for its ability to inhibit inflammation in RAW 264.7 Macrophage by suppressing the production of PGE2 and NO, and downregulating TNF-α, IL-6, IL-1β and NF-κB/MAPK expressions (Lee et al. 2021; Nagahawatta et al. 2022). *Ecklonia maxima* polysaccharides have also been reported for their potent antioxidant and neuroprotective properties (Olasehinde et al. 2019a).

Storage conditions play influential role on the safety, quality and sensory characteristics of food products (Calligaris et al. 2019). It affects the degradation mechanism of the product which is influenced by heat, light, temperature, enzymes, chemicals and microbial contamination (Azanha and Faria 2005). Under frozen conditions, degradation is triggered by oxidative, enzymatic reactions, surface drying and re-crystallization (Calligaris et al. 2019). At ambient conditions, degradation often occurs from chemical reactions, such as lipid oxidation, flavor degradation, and pigment alteration, as well as non-enzymatic browning and physical changes including starch retrogradation and product structural collapse (Calligaris et al. 2019).

The stability of polysaccharides during storage has been reported to be affected by storage conditions, and it is characterized by changes in their texture and flavor, chemistry and biological activities (Feng et al. 2020; Peroni-Okita et al. 2013). These changes are influenced by temperature and storage conditions, and occurs via various mechanisms such as retrogradation (Feng et al. 2020), enzymatic hydrolysis (Yu et al. 2022), freezing and thawing (Feng et al. 2020), coldinduced

sweetening (Peroni-Okita et al. 2013; Yu et al. 2022), acid hydrolysis (Keran et al. 2025), thermal degradation (Silva et al. 2025) and UV irradiation (Song et al. 2024). The changes in biological activity include fluctuating antioxidant, antidiabetic and antiobesogenic activities, with different studies reporting contrasting results of improved (Song et al. 2024; Zhao et al. 2023; Zou et al. 2020) and reduced (Radzki et al. 2016; Zou et al. 2020) activities.

Despite these studies, there is a dearth of information on the effect storage conditions on the stability, chemical profiles and biological activities of sulphated polysaccharides from brown and red seaweeds. Thus, the present study was undertaken to investigate the effect of storage conditions on the stability of *E. maxima* and *G. gracilis* SPs vis-à-vis their chemical properties and antidiabetic activities.

5.2 Materials and Methods

Kindly refer to sections 2.1 – 2.3 in Chapter 2.

5.3. Results and Discussion

5.3.1 Effect of storage on glucose and sulphate content of sulfated polysaccharides

Tables 5.1 and 5.2 revealed the total sugar and sulphate content of polysaccharides stored at different temperature after storage at different interval. No significant changes were observed in the glucose levels after the first and second month when compared to the baseline as shown in Table 5.1. However, at 37 °C, there was a significant ($p<0.05$) reduction in glucose levels following storage for 3-4 months. Further significant ($p<0.05$) reduction was also observed after the 5th and 6th month at the same temperature. A slight decrease was also observed after the SPs were stored at 25 °C especially after the 6th month as shown in Table 5.1. Overall, stability of glucose level was

maintained at 4 °C and 25 °C. Also, our findings also revealed that over 60 % of glucose levels was retained in SPs stored after 6 months at 37 °C as shown by glucose level obtained in Table 5.1. Reduced total glucose concentrations in polysaccharides has been implicated in physiochemical alteration including changes in physical properties like viscosity and gelling behaviour, as well as altered reactivity in chemical reactions like the Maillard reaction (Li et al. 2019; Lovegrove et al. 2017). Thus, the reducing glucose concentrations of the SPs, with increasing storage temperature and time, indicates alteration in their physiochemical properties. These alterations are temperature and time dependent.

Table 5.1: Glucose levels (mg/mL) of Sulphated Polysaccharides at different storage temperatures and time

Seaweed	**Baselines**		
G. gracilis SPs	0.060 ± 0.010^{b}		
E. maxima SPs	0.067 ± 0.013^{b}		
	Month 1		
	4°C	**25°C**	**37°C**
G. gracilis SPs	0.048 ± 0.011^{a}	0.049 ± 0.010^{a}	0.039 ± 0.009^{a}
E. maxima SPs	0.052 ± 0.019^{a}	0.050 ± 0.007^{a}	0.048 ± 0.012^{a}
	Month 2		
G. gracilis SPs	0.057 ± 0.010^{a}	0.047 ± 0.04^{a}	0.038 ± 0.006^{b}
E. maxima SPs	0.044 ± 0.012^{a}	0.054 ± 0.010^{a}	0.051 ± 0.011^{a}
	Month 3		
G. gracilis SPs	0.057 ± 0.001^{a}	0.058 ± 0.029^{a}	0.044 ± 0.002^{b}
E. maxima SPs	0.067 ± 0.005^{a}	0.051 ± 0.024^{a}	0.035 ± 0.004^{b}
	Month 4		
G. gracilis SPs	0.050 ± 0.012^{a}	0.051 ± 0.018^{a}	0.041 ± 0.005^{a}
E. maxima SPs	0.061 ± 0.009^{a}	0.047 ± 0.012^{a}	0.030 ± 0.003^{b}
	Month 5		
G. gracilis SPs	0.053 ± 0.007^{a}	0.051 ± 0.005^{a}	0.033 ± 0.002^{b}

E. maxima SPs	0.059 ± 0.004[a]	0.045 ± 0.008[a]	0.031 ± 0.004[b]
	Month 6		
G. gracilis SPs	0.049 ± 0.008[a]	0.038 ± 0.005[b]	0.028 ± 0.003[c]
E. maxima SPs	0.050 0.005[a]	0.040 ± 0.003[b]	0.030 ± 0.001[c]

Values = mean□SD; n=3. *[a,b,c] Values with different letters above the bars are significantly ($p<0.05$) different from each other.

Similarly, stability of sulphate levels was assessed at different stored temperatures (4 °C, 25 °C and 37 °C) for 6 months. Our findings revealed that the sulphate levels of the SPs were stable especially at 4 °C and 25 °C within the 6 months interval. A significant ($P< 0.05$) reduction in sulphate levels in the SPs stored at 37 °C was observed after the 5th and 6th month. Overall, these findings showed that the stability of sulphate levels were maintained in the SPs at 4 °C and 25 °C. At C, there was no reduction of sulphate levels in the three SPs even at 6 months of storage This means 100 % of the sulphate content was retained. Similar results were obtained in SPs stored at 25 °C. The percentage of subplate levels retained after 6 months at 25 °C, were 78 % and 85 % in *G. gracilis* and *E. maxima* SPs, respectively. Also, at 37 °C percentage of sulphate retained in *G. gracilis* and *E. maxima* were 64.2 %, 53.3 and 30 %, respectively. These results revealed that the SPs may be best maintained at 4 and 25 °C. The alteration in sulphate levels, further suggests instability in the physicochemical properties of the SPs. Sulphur contributes to the solubility of polysaccharides and decreases their viscosity, while enhancing their surface area and flexibility (Bae et al. 2009; Gunasekaran et al. 2021).

Table 5.2: Sulphate levels (mg/mL) of Sulphated Polysaccharides at different storage temperatures and time

Seaweed	Baselines
G. gracilis SPs	0.050 ± 0.004[b]
E. maxima SPs	0.153 ± 0.012[b]

	4ºC	25ºC	37ºC
Month 1			
G. gracilis SPs	0.083 ± 0.011[b]	0.054 ± 0.007[a]	0.050 ± 0.020[a]
E. maxima SPs	0.150 ± 0.009[a]	0.157 ± 0.007[a]	0.146 ± 0.010[a]
Month 2			
G. gracilis SPs	0.055 ± 0.010 [a]	0.051± 0.013 [a]	0.047 ±0.006 [a]
E. maxima SPs	0.149 ± 0.011 [a]	0.155 ± 0.010 [a]	0.124 ± 0.008[b]
Month 3			
G. gracilis SPs	0.045 ± 0.002 [a]	0.049±0.004 [a]	0.041± 0.001 [a]
E. maxima SPs	0.145 ± 0.008 [a]	0.145 ± 0.006 [a]	0.120 ± 0.005[b]
Month 4			
G. gracilis SPs	0.051 ± 0.005 [a]	0.044 ± 0.002 [a]	0.035 + 0.002[b]
E. maxima SPs	0.146 ± 0.009 [a]	0.141 ± 0.001 [a]	0.111 ± 0.002[b]
Month 5			
G. gracilis SPs	0.047 ± 0.003 [a]	0.040 ± 0.001[b]	0.028 ± 0.004[b]
E. maxima SPs	0.141 ± 0.009 [a]	0.137 ± 0.003 [a]	0.094 ± 0.002[b]
Month 6			
G. gracilis SPs	0.050 ± 0.002 [a]	0.035 ± 0.001[b]	0.020 ± 0.002[c]
E. maxima SPs	0.143 ± 0.005 [a]	0.130 ± 0.004[b]	0.081 ± 0.005[b]

Values = mean SD; n=3. [a,b,c] Values with different letters above the bars are significantly ($p<0.05$) different from each other**.**

Carbohydrate hydrolysing enzymes such as α-glucosidase and α-amylase play an important role in the control of postprandial glucose. In diabetic conditions, the activity of these enzymes are significantly high thereby causing the rapid release of glucose into the blood stream which contributes to hyperglycemia. Hence, inhibiting α-glucosidase and α-amylase reduce the release of glucose from the intestine into the blood stream thereby mitigating hyperglycemia.

Figures 5.1A and 5.1B show the α-glucosidase inhibitory activities of *G. gracilis* and *E. maxima* SPs stored at different temperatures and time intervals. Storage at 4 °C and 25 °C, caused no significant ($P > 0.05$) changes in the activities of the SPs. However, there was a significant ($p<0.05$)

decrease in α-glucosidase activity of SPs stored at 37 °C at different time interval. Similarly in Figures 5.2A and 5.2B, the SPs stored at different temperatures and intervals significantly ($P<0.05$) inhibited α-amylase activity. The inhibitory activity of the SPs was maintained at 4 °C and 25 °C. However, a significant ($p<0.05$) decrease in the inhibitory activity was observed for SPs stored at 37 °C.

These results revealed that the activity of the SPs was maintained at 4 °C and 25 °C which correlates with the results obtained from the glucose and sulphate contents. These suppressed α-glucosidase and α-amylase activities at 37 °C, suggest decreased ability of the SPs to inhibit carbohydrate digestion, which can lead to postprandial rise in blood glucose level (Zeng et al. 2025). This corresponds with previous studies on decreased α-glucosidase activity with increasing temperature (Da Silva et al. 2009). The decreased activities can be attributed to altered physicochemical properties. The antidiabetic activity of sulphated polysaccharides has been linked to the type of glycosidic bond, monosaccharide composition, and sulphate contents. Zhang et al. (2024) reported that the monosaccharide composition of sulfated polysaccharides contributed to the antidiabetic activities of polysaccharides. The work of Koh et al. (2020) also showed that the α-glucosidase and α-amylase inhibitory effects of sulfated polysaccharides from *Undiaria pinnatifida* is dependent on sulphate contents. Hence, the observed α-glucosidase and α-amylase inhibitory effects exerted by the SPs used in this study and stored at different temperatures may be linked to their sulfate contents and monosaccharide composition. The monosaccharide composition of the SPs used in this study have been reported previously Erukainure et al. (2019). Also, the reduced α-glucosidase and αamylase inhibitory effects of SPs stored at 37 °C may be due to the reduction of the sulfate content.

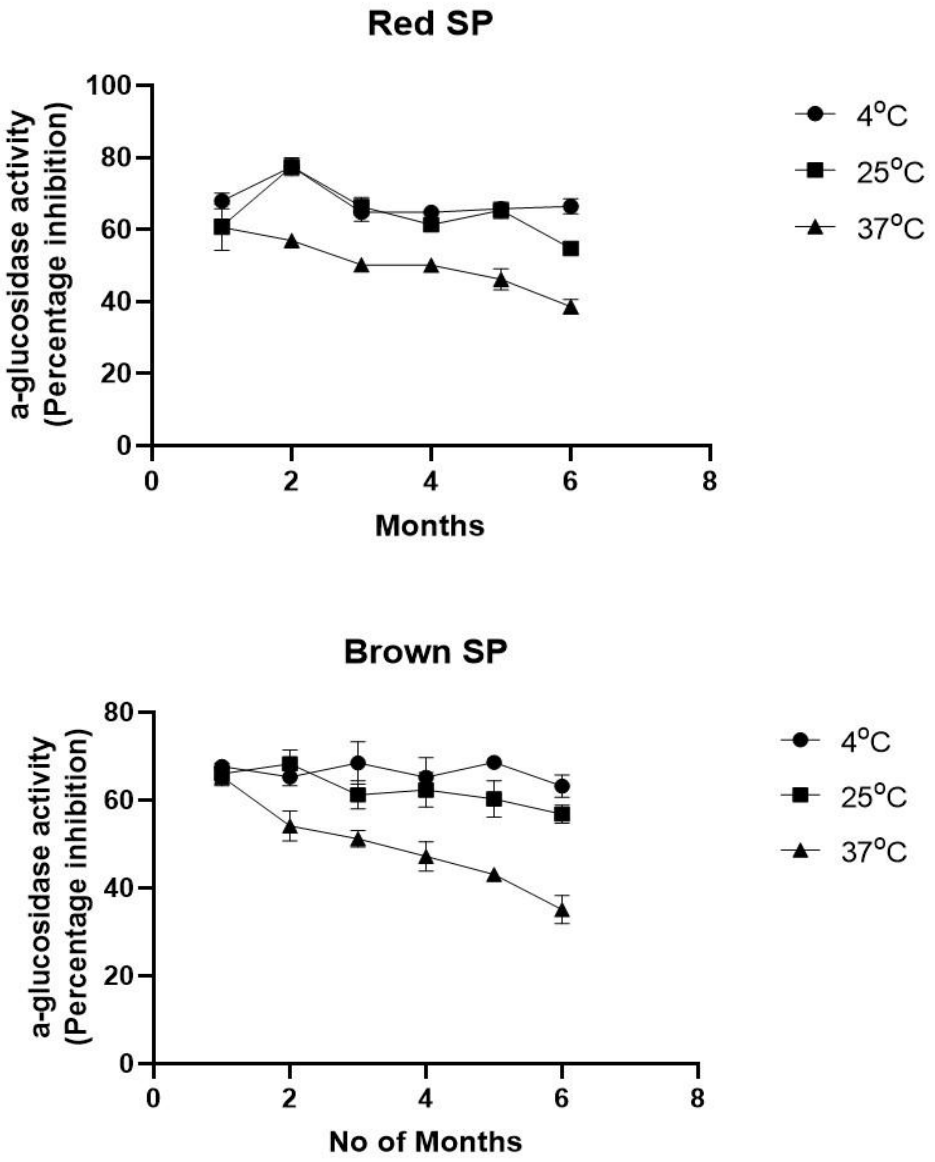


Figure 5.1: Effect of storage temperature and time on α-glucosidase activities of (A) *G. gracilis* and (B) *E. maxima* SPs. Values = mean□SD; n=3.

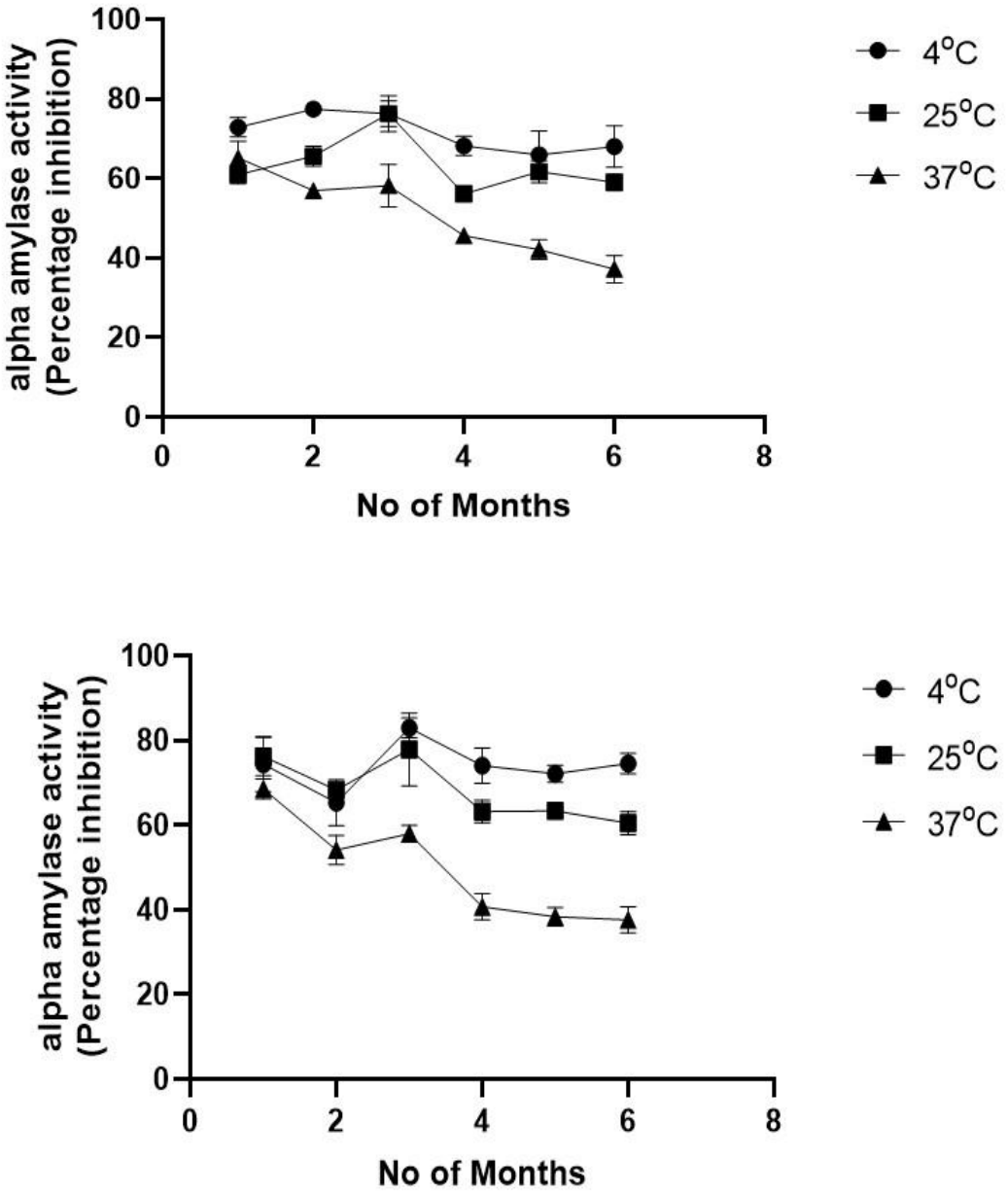


Figure 5.2: Effect of storage temperature and time on α-amylase activities of (A) *G. gracilis* and (B) *E. maxima* SPs. Values = mean□SD; n=3.

5.4. Conclusion

These results suggest a temperature dependent instability and decreased antidiabetic activities of SPs from *G. gracilis* and *E. maxima* which is influenced by storage time. This is depicted by decreased glucose and sulphate levels, and suppressed activities of α-glucosidase and α-amylase

activities. However, there is need to study other attributes of shelf-life such as microbial contamination, heat, light, and pH that may affect the stability of the SPs.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported by a grant from Technology Innovation Agency (TIA), Durban, South Africa (Grant no. SABDI 16/1072), and Incentives for Rated Researchers by the National Research Foundation (NRF).

Data availability statement

The data in support this research findings are presented in the article.

5.5 Reference

Azanha A, Faria J (2005) Use of mathematical models for estimating the shelf-life of cornflakes in flexible packaging. Packaging Technology and Science: An International Journal 18(4):171– 178

Bae IY, Joe YN, Rha HJ, Lee S, Yoo S-H, Lee HG (2009) Effect of sulfation on the physicochemical and biological properties of citrus pectins. Food Hydrocolloids 23(7):1980–1983

Borazjani NJ, Tabarsa M, You S, Rezaei M (2018) Purification, molecular properties, structural characterization, and immunomodulatory activities of water soluble polysaccharides from Sargassum angustifolium. International journal of biological macromolecules 109:793–

802 Calligaris S, Manzocco L, Anese M, Nicoli MC (2019) Accelerated shelf life testing Food quality and shelf life. Elsevier, p 359–392

Da Silva TM, Michelin M, de Lima Damásio AR, et al. (2009) Purification and biochemical characterization of a novel α-glucosidase from Aspergillus niveus. Antonie van Leeuwenhoek 96:569–578

Daub CD, Mabate B, Malgas S, Pletschke BI (2020) Fucoidan from Ecklonia maxima is a powerful inhibitor of the diabetes-related enzyme, α-glucosidase. International journal of biological macromolecules 151:412–420 de Jesus Raposo MF, De Morais AMB, De Morais RMSC (2015) Marine polysaccharides from algae with potential biomedical applications. Marine drugs 13(5):2967–3028

Feng W, Ma S, Wang X (2020) Recent advances in quality deterioration and improvement of starch in frozen dough. Grain & Oil Science and Technology 3(4):154–163

Gunasekaran S, Govindan S, Ramani P (2021) Sulfated modification, characterization and bioactivities of an acidic polysaccharide fraction from an edible mushroom Pleurotus eous (Berk.) Sacc. Heliyon 7(1)

Hentati F, Tounsi L, Djomdi D, et al. (2020) Bioactive polysaccharides from seaweeds. Molecules 25(14):3152

Jiang H, Yang SQ, Chakka VP, et al. (2021) Purification and biological activities of enzymatically degraded Sargassum fusiforme polysaccharides. Chemistry & Biodiversity 18(3):e2000930

Keran DA, Kumar PS, Venugopal V, Kumar R, Tarakanth K, Chinnappa A (2025) Degradation of polysaccharides during food processing Chemistry of Thermal and Non-Thermal Food Processing Technologies. Elsevier, p 461–501

Khandwal D, Patel S, Pandey AK, Mishra A (2025) A Comprehensive, Analytical Narrative Review of Polysaccharides from the Red Seaweed Gracilaria: Pharmaceutical Applications and Mechanistic Insights for Human Health. Nutrients 17(5):744

Kumar Bhateja P, Singh R (2014) Antidiabetic Activity of Acacia Tortilis (Forsk.) Hayne Ssp. Raddiana Polysaccharide on Streptozotocin-Nicotinamide Induced Diabetic Rats. BioMed research international 2014(1):572013

Lee H-G, Je J-G, Hwang J, et al. (2021) Comparision of antioxidant and anti-inflammatory activities of enzyme assisted hydrolysate from Ecklonia maxima blades and stipe. Fisheries and Aquatic Sciences 24(5):197–206

Li X, Dong Y, Guo Y, et al. (2019) Okra polysaccharides reduced the gelling-required sucrose content in its synergistic gel with high-methoxyl pectin by microphase separation effect. Food Hydrocolloids 95:506–516

Lovegrove A, Edwards C, De Noni I, et al. (2017) Role of polysaccharides in food, digestion, and health. Critical reviews in food science and nutrition 57(2):237–253

Murakami S, Hirazawa C, Ohya T, et al. (2021) The edible brown seaweed Sargassum horneri (Turner) C. Agardh ameliorates high-fat diet-induced obesity, diabetes, and hepatic steatosis in mice. Nutrients 13(2):551

Nagahawatta D, Liyanage N, Jayawardhana H, Lee H-G, Jayawardena TU, Jeon Y-J (2022) Antifine dust effect of fucoidan extracted from Ecklonia maxima leaves in macrophages via inhibiting inflammatory signaling pathways. Marine Drugs 20(7):413

Olasehinde TA, Mabinya LV, Olaniran AO, Okoh AI (2019a) Chemical characterization, antioxidant properties, cholinesterase inhibitory and anti-amyloidogenic activities of sulfated polysaccharides from some seaweeds. Bioactive Carbohydrates and Dietary Fibre 18:100182

Olasehinde TA, Olaniran AO, Okoh AI (2019b) Macroalgae as a valuable source of naturally occurring bioactive compounds for the treatment of Alzheimer's disease. Marine drugs 17(11):609

Pereira R, Yarish C (2008) Mass production of marine macroalgae In: Jørgensen, SE, Fath, BD (Eds.), Encyclopedia of Ecology. Academic Press, Oxford

Peroni-Okita FH, Cardoso MB, Agopian RG, et al. (2013) The cold storage of green bananas affects the starch degradation during ripening at higher temperature. Carbohydrate polymers 96(1):137–147

Pillay LR, Olasehinde TA, Olofinsan K, et al. (2025) The impact of sulphated polysaccharides from Ecklonia maxima on carbohydrate hydrolyzing enzymes, muscle glucose uptake and intestinal glucose absorption ex vivo. Algal Research 85:103831

Pillay LR, Olasehinde TA, Olofinsan KA, Erukainure OL, Islam MS, Olaniran AO (2024) Antidiabetic potentials of crude and purified sulphated polysaccharides isolated from Gracilaria gracilis, a seaweed from South Africa. Heliyon 10(15)

Radzki W, Ziaja-Sołtys M, Nowak J, et al. (2016) Effect of processing on the content and biological activity of polysaccharides from Pleurotus ostreatus mushroom. LWT-Food Science and Technology 66:27–33

Silberfeld T, Leigh JW, Verbruggen H, Cruaud C, De Reviers B, Rousseau F (2010) A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): Investigating the evolutionary nature of the “brown algal crown radiation”. Molecular phylogenetics and evolution 56(2):659–674

Silva M, Vida M, Ramos AC, Lidon FJ, Reboredo FH, Gonçalves EM (2025) Storage Temperature Effect on Quality and Shelf-Life of Hericium erinaceus Mushroom. Horticulturae 11(2):158

Song P, Huang Y, Li J, et al. (2024) The influence of processing technologies on the biological activity of carbohydrates in food. Food Chemistry: X 23:101590

Yu J, Tseng Y, Pham K, Liu M, Beckles DM (2022) Starch and sugars as determinants of postharvest shelf life and quality: some new and surprising roles. Current opinion in biotechnology 78:102844

Zeng X-X, Wang L, Wang M-Y, et al. (2025) BuZhong YiQi Formula Alleviates Postprandial

Hyperglycemia in T2DM Rats by Inhibiting α-Amylase and α-Glucosidase In Vitro and In Vivo. Pharmaceuticals 18(2):201

Zhao Z, Wang L, Ruan Y, et al. (2023) Physicochemical properties and biological activities of polysaccharides from the peel of Dioscorea opposita Thunb. extracted by four different methods. Food Science and Human Wellness 12(1):130–139

Zhou Q-L, Wang Z, Chen W-T, et al. (2024) The structural characteristics, biological activities and mechanisms of bioactive brown seaweed polysaccharides: A review. Journal of Functional Foods 119:106303

Zou M-Y, Nie S-P, Yin J-Y, Xie M-Y (2020) Ascorbic acid induced degradation of polysaccharide from natural products: a review. International Journal of Biological Macromolecules 151:483–491

CHAPTER 6 General Discussions and Conclusions

6.1 General Discussion

Diabetic hepatopathy is among the microvascular complications of T2D (Mallet et al. 2022; Tolman et al. 2007), with hyperglycemia, dyslipidemia, insulin resistance and hyperinsulinemia playing major roles (Mobasheri et al. 2023). It is a spectrum of hepatic diseases that includes NAFLD, hepatocellular carcinoma, abnormal liver enzymes, cirrhosis, and acute liver failure (Islam et al. 2020; Mobasheri et al. 2023; Tolman et al. 2007). There has been increasing paradigm shift towards the use of natural products such as polysaccharides in the management of diabetic hepatopathy. In the present study, we investigated the effect of SPs from the seaweeds, *Ecklonia maxima* and *Gracilaria gracilis*, on diabetic hepatopathy in livers of rats with T2D. We employed biochemical and metabolomics approaches in deciphering their hepatoprotective mechanism. Furthermore, the effect of storage conditions and time on the stability of SPs and their antidiabetic activities were investigated in *in vitro* models.

Altered glucose metabolism, characterized by incessant hepatic gluconeogenesis and glycogenolysis, has been implicated in the pathogenesis of diabetic hepatopathy as they contribute to elevated blood glucose (Barroso et al. 2024; Zhang et al. 2019). SPs from both seaweeds mitigated gluconeogenesis and glycogenolysis in the present study, by suppressing the activities of fructose-1,6-biphosphatase, glucose 6-phosphatase and glycogen phosphorylase (Figures 3.1 and 4.1). Furthermore, the SPs upregulated hepatic citric acid cycle, malate-aspartate shuttle and Warburg effect.

As reported in chapters 3 and 4, induction of T2D elevated hepatic. oxidative stress and inflammation by increasing MDA level and acetylcholinesterase activity, while concomitantly

suppressing SOD, catalase, glutathione reductase, glutathione peroxidase, GST activities and GSH level. These results corroborated previous studies on the induction of hepatic oxidative injuries in T2D (Klisic et al. 2018; Zhang et al. 2012). Both SPs from *E. maxima* and *G. gracilis* mitigated the oxidative stress as depicted by their ability to reverse the activities and levels of the oxidative stress biomarkers (Figures 3.2, 3.3, 4.2 and 4.3). These antioxidant and antiinflammatory activities corroborate previous studies on the therapeutic effect of antioxidants in managing hepatic oxidative stress (Bae et al. 2023).

Induction of T2D led to the distortions of hepatic lipid metabolism and its metabolites, indicating an occurrence of hyperlipidemia and lipotoxicity. These distortions have been implicated in the pathogenesis and progression of diabetic hepatopathy (Gluchowski et al. 2017). The ability of both SPs from *E. maxima* and *G. gracilis* to reverse diabetic-upregulated hepatic lipid metabolic pathways, particularly oxidation of branched chain fatty acids, phytanic acid peroxisomal oxidation, and mitochondrial beta-oxidation of long chain saturated fatty acids pathways (Figures 3.6, 3.7B, 4.7, 4.8B and Tables 3.2 and 4.2), indicates their antilipidemic effect. Further, suggesting the hepatoprotective effect of the polysaccharides in the management of diabetic hepatopathy.

Dysregulation of hepatic amino acids metabolism leading to altered blood concentrations of essential amino acids, such as BCAAs, tyrosine, phenylalanine, and sulfur amino acids, has been reported in the pathology and progression of diabetic hepatopathy (Adams 2011; Cuomo et al. 2022; Wiklund et al. 2018). The ability of both SPs from *E. maxima* and *G. gracilis* to mitigate the dysregulated hepatic amino acid metabolism in T2D rats (Figure 3.7C and 4.8C, and Tables 3.2 and 4.2), suggests their therapeutic potentials against diabetic hepatopathy.

The conditions at which polysaccharides are stored are crucial in the maintenance of their stability, phytochemistry and biological activities. In chapter 5, storage of SPs from *E. maxima* and *G. gracilis* at 4 and 25 □C, had no significant effect on their glucose and sulphate levels (Tables 5. 1

and 5.2), as well as their inhibition of □-glucosidase, and □-amylase inhibitory activities. However, storage at 37□C showed significant reduction in the SPs glucose and sulphate levels, at the 5th and 6th month. Thus, indicating a time and temperature dependent deteriorative effect. The □-glucosidase, and □-amylase inhibitory activities declined following storage at 37□C, and continued with increasing time.

6.2 Conclusions

These results indicate the hepatoprotective effect of SPs from *E. maxima* and *G. gracilis* on diabetic hepatopathy in T2D. This is depicted by their ability to mitigate oxidative stress, inflammation and lipotoxicity, while improving glucose and amino acid metabolisms. Furthermore, the stability of the SPs and their antidiabetic activities may be temperature dependent, influenced by storage time.

6.3. Recommendations

Although these results show promising effect of SPs in the management of diabetic hepatopathy, there is need to explore the translational relevance of these therapeutic potentials in clinical trials. Furthermore, other attributes of shelf-life such as microbial contamination, heat, light, and pH that may contribute to the instability of the SPs, need to be investigated.

6.4. References

Adams SH (2011) Emerging perspectives on essential amino acid metabolism in obesity and the insulin-resistant state. Advances in nutrition 2(6):445–456

Bae C-S, Lee Y, Ahn T (2023) Therapeutic treatments for diabetes mellitus-induced liver injury by regulating oxidative stress and inflammation. Applied Microscopy 53(1):4

Barroso E, Jurado-Aguilar J, Wahli W, Palomer X, Vázquez-Carrera M (2024) Increased hepatic gluconeogenesis and type 2 diabetes mellitus. Trends in Endocrinology & Metabolism

Cuomo P, Capparelli R, Iannelli A, Iannelli D (2022) Role of branched-chain amino acid metabolism in type 2 diabetes, obesity, cardiovascular disease and non-alcoholic fatty liver disease. International journal of molecular sciences 23(8):4325

Gluchowski NL, Becuwe M, Walther TC, Farese Jr RV (2017) Lipid droplets and liver disease: from basic biology to clinical implications. Nature reviews Gastroenterology & hepatology 14(6):343–355

Islam S, Rahman S, Haque T, Sumon AH, Ahmed AM, Ali N (2020) Prevalence of elevated liver enzymes and its association with type 2 diabetes: A cross-sectional study in Bangladeshi adults. Endocrinology, diabetes & metabolism 3(2):e00116

Klisic A, Isakovic A, Kocic G, et al. (2018) Relationship between oxidative stress, inflammation and dyslipidemia with fatty liver index in patients with type 2 diabetes mellitus. Experimental and Clinical Endocrinology & Diabetes 126(06):371–378

Mallet V, Parlati L, Martinino A, et al. (2022) Burden of liver disease progression in hospitalized patients with type 2 diabetes mellitus. Journal of Hepatology 76(2):265–274

Mobasheri L, Ahadi M, Namdar AB, et al. (2023) Pathophysiology of diabetic hepatopathy and molecular mechanisms underlying the hepatoprotective effects of phytochemicals. Biomedicine & Pharmacotherapy 167:115502

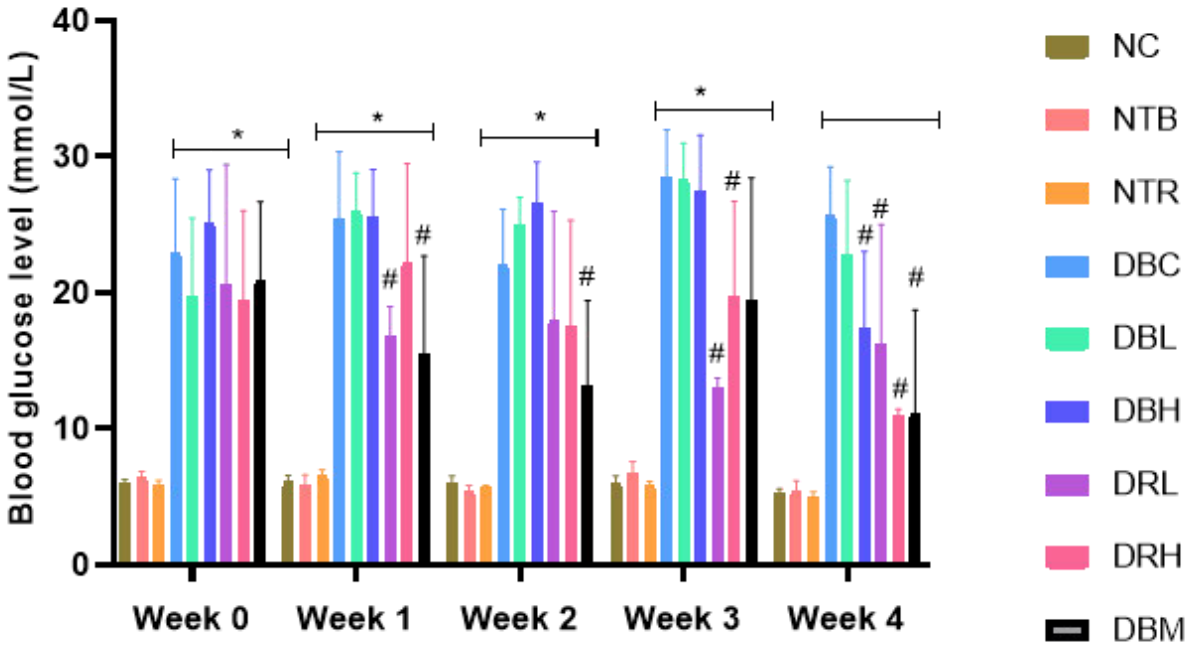
Tolman KG, Fonseca V, Dalpiaz A, Tan MH (2007) Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. Diabetes care 30(3):734–743

Wiklund P, Zhang X, Pekkala S, Autio R, Kong L, Yang Y (2018) Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. Sci Rep. 2016; 6: 1–11. American Journal of Epidemiology 187(8):1662–1669

Zhang C, Lu X, Tan Y, et al. (2012) Diabetes-induced hepatic pathogenic damage, inflammation, oxidative stress, and insulin resistance was exacerbated in zinc deficient mouse model. PloS one 7(12):e49257

Zhang X, Yang S, Chen J, Su Z (2019) Unraveling the regulation of hepatic gluconeogenesis. Frontiers in Endocrinology 9:802

Appendix



Appendix 1. Blood glucose levels of normal, untreated and treated diabetic rats. Data = mean ± SD; n =6.